THE INHERITANCE OF FLOWERING TIME IN A SHORT-DAY SPECIES, SOLIDAGO SEMPERVIRENS L.[1]

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INTRODUCTION

OBSERVATIONS on the differences in flowering time of various strains of the seaside golden-rod, *Solidago sempervirens* L., have now been made over a period of several years. This species is a fall-flowering perennial which, in its native habitat, flowers progressively later in the season as one passes from north to south along the Atlantic Coast. When plants from different portions of the range have been grown under cultivation at the latitude of Rochester, New York, these plants still flower at different times, those from the north flowering earlier than those from the south. This has been interpreted as evidence for the existence within this species of a graded series of strains genetically distinguishable from one another by their physiological requirements for floral initiation (GOODWIN 1941). A very similar situation has been described by TURESSON (1930) for the European golden-rod, *S. virgaurea* L., but there altitude is a complicating factor, early-flowering strains being found at high altitudes even in the southerly portions of the range.

A large number of species and strains within species commence to flower in a given locality at a very definite time in the calendar year, a certain amount of variation being due to minor climatic fluctuations. For example, the regularity with which the three strains of *S. sempervirens* studied in the present investigation have flowered at Rochester for the past four years is shown in table 1. Through the work of GARNER and ALLARD (1920) and subsequent investigators, it is now known that the principal environmental factor controlling the initiation of floral primordia in many species of plants is the photoperiod. Numerous fall-flowering perennials have been shown to be "short-day" plants,

TABLE 1

Mean dates for anthesis of the first disc florets of the inflorescence in three strains of S. sempervirens at Rochester, New York. Dates in italics are for plants grown outdoors. All other dates are for plants grown in the greenhouse.

YEAR	MASS.	MD.	FLA.
1940	*Sept. 18*	*Oct. 4*	Nov. 16
1941	*Sept. 16*	*Oct. 4*	
1942	Sept. 14	Sept. 27	Oct. 30
1943	Sept. 15	Sept. 28	Nov. 13

[1] It is a pleasure to acknowledge the advice and encouragement generously given by DR. DONALD R. CHARLES during the course of this investigation. Thanks are also due to MR. WILLIAM STEPKA and MR. FREDERICK C. UFFELMAN for their assistance in growing the plants.

which require a diurnal dark period of a certain minimal duration before passing into the reproductive phase of development. *S. sempervirens*, among other species in the genus, has been shown to be a short-day plant. The flowering of a strain from Connecticut has been hastened experimentally by at least three months by exposing vegetative plants to artificially shortened photoperiods (ALLARD and GARNER 1940), and a strain from Florida has also responded to a similar treatment (see this paper). The various genetic strains of this species presumably differ in their flowering time because they differ also in their physiological responses to the ever-shortening photoperiod (see fig. 1), the later the strain, the longer the critical dark period necessary for floral initiation.

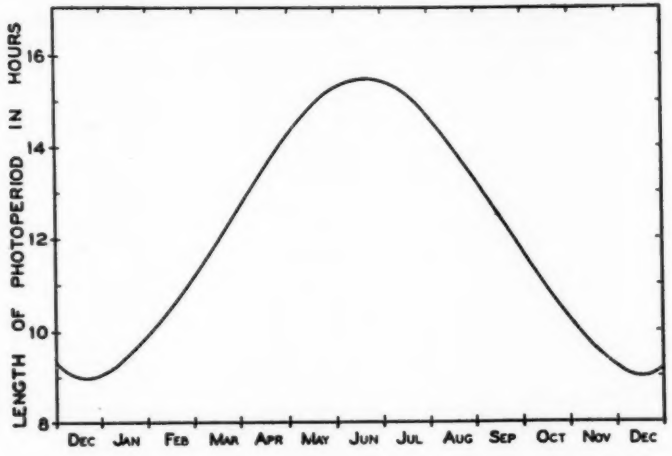

FIG. 1.—The number of hours between sunrise and sunset at Rochester, New York. The data upon which this curve is based were obtained by interpolation from tables given in the American Nautical Almanac for 1943. The photoperiod actually controlling the flowering behavior of *S. sempervirens* may be slightly longer than that given here, since the light intensity for a short time before sunrise and after sunset is sufficiently great to have a photoperiodic effect upon certain other species

Among the angiosperms, early and late-flowering strains are now known to occur in a great many species. Numerous investigations on the inheritance of flowering time have been made; but most of the cases reported have involved cultivated plants in which the initiation of flower primordia in a given strain under a given set of environmental conditions occurs a definite number of days after germination—for example, peas (KEEBLE and PELLEW 1910; HOSHINO 1915; RASMUSSON 1935), sweet peas (LITTLE and KANTOR 1941), flax (BARTELS 1940), cotton (LEAKE 1911), tomatoes (POWERS and LYON 1941), wheat (THOMPSON 1918; FREEMAN 1919; FLORELL 1924), barley (KUCKUCK 1930), and rice (HOSHINO 1915). Genetic studies have demonstrated the mendelian nature of this inheritance. In certain instances genes may be dominant (LITTLE and KANTOR 1941), but more frequently heterozygosity results in a more or

less intermediate condition (RASMUSSON 1935), and inheritance appears to be of a "quantitative" type. These genetic studies have not dealt with short-day species, in which the flowering time is primarily controlled by the length of the photoperiod, although the photoperiod is doubtless one of the environmental factors involved in certain cases.

In cultivated tobacco (*Nicotiana Tabacum*), normally a long-day plant, mammoth varieties occur which flower only under short-day conditions. Crosses between mammoth and regular long-day varieties suggest that a single recessive gene for short-day flowering behavior is primarily responsible for the development of mammoth strains (ALLARD 1919).

The present investigation represents a contribution toward an understanding of the inheritance of responses to the photoperiod in three strains of *S. sempervirens*. An attempt has been made to distinguish between floral initiation and the subsequent development of the floral primordia and to estimate the minimal number of genes which must be involved in differentiating these strains with respect to these two developmental characters.

MATERIALS AND METHODS

Three strains of *S. sempervirens* L. were used in this investigation. One, which will be referred to as the "Mass." strain, was derived from cross pollinations of specimens of the typical species collected by the author in 1934 on the sand dunes at Ipswich, Massachusetts. The other two strains belong to the var. *mexicana* (L.) Fernald. Of these, the "Md." strain was transplanted by the author in 1934, from a sandy, vacant lot behind the beach at Ocean City, Maryland, and the "Fla." strain was grown from seed collected in the same year by MR. W. M. BUSWELL at Ft. Myers, Florida.

It has been previously pointed out (GOODWIN 1941) that these strains differ in their flowering behavior. In table 1 the mean dates of anthesis of the first disc florets during the past four years at Rochester, New York, are given for these three strains.

Earlier investigations (GOODWIN 1937) have shown that *S. sempervirens*, among other species of golden-rod, is self sterile. Inflorescences which have been protected from insect pollination with fine gauze bags before the opening of the buds do not produce viable seeds unless artificially cross-pollinated. Hybridizations were made, therefore, by cross-pollinating bagged inflorescences.

In order to obtain simultaneous flowering of the early and late strains for hybridizing, the reproductive cycle of plants of the Fla. strain was hastened by covering them with hoods of heavy black cloth from late afternoon until early morning, thereby shortening the natural photoperiod to a 9- to 10-hour day. This treatment was begun in late August, 1941, and the following crosses were made successfully between October 6 and 19 of that year: Mass.×Fla. and Md.×Fla. F_1 generations were grown from the seed obtained from these crosses, and F_2 hybrids and backcrosses were successfully produced in the fall of 1942.

In the spring of 1943 seeds for the F_2 hybrid and backcross populations were germinated on filter paper in Petri dishes at 30°C from February 6 to 19. Germi-

nating seedlings were planted individually in two and one-half inch pots and were periodically repotted. By mid-June all seedlings were in six-inch pots—the largest size used. Meanwhile, plants of the parental strains and of the F_1 hybrid populations, which had been grown in 1942, were kept in a dormant condition throughout the winter months in a cool section of the greenhouse. In April and early May these plants were repotted in six-inch pots, at which time their root systems were thinned and their shoots were reduced to two or three vigorous rosettes. In this way populations of one-year-old and two-year-old plants were obtained which appeared strictly comparable by mid-June. *S. sempervirens* is a perennial which normally produces a sterile rosette during the first growing season and flowering axes only in subsequent years. If seedlings are given an early start by germinating them in the greenhouse in February or March, however, they may be brought into flower during the first

TABLE 2

A comparison of dates of anthesis of the first disc florets for the Mass. strain in 1942 and 1943. Standard deviations are given.

	NUMBER OF PLANTS	DATE OF ANTHESIS (SEPT.) 1942	1943
Av. for all plants flowering in 1942	12	13.7±5.5	
Av. for all plants flowering in 1943	19		15.5±5.7
Av. for plants germinated in 1942 and flowering in 1942 and 1943	9	14.0±5.7	16.0±6.7
Av. for all plants germinated in 1942, flowering in 1943	15		16.1±6.1
Av. for plants germinated in 1943	4		13.0±2.4

growing season, and flowering will take place at essentially the same time for these young plants as for older plants of the same genetic constitution (see table 2).

Data on the size and behavior of the various populations studied during the summer and fall of 1943 are given in table 3. There was a loss of seedlings during the early-post-germination stages due to a variety of causes, but the percentage loss was small, except in the F_2 population of the Mass.×Fla. cross. In this case, 74 plants (44 percent) failed to survive. Of these, a majority stopped growing by the end of the first week after germination, became chlorotic, and eventually died. It is significant that this large loss occurred in the inter-varietal cross between the typical species and its southern variety (Mass.×Fla.), where the greatest genetic incompatability might be expected, and not in the intra-varietal cross (Md.×Fla.). A certain number of the survivors in the various populations remained in the rosette stage (failed to produce an elongated axis) for the entire season and never flowered. Of those plants which

TABLE 3

Some statistics on the size and behavior of the populations studied.

POPULATION	NUMBER OF PLANTS GERMINATED IN 1943	NUMBER OF PLANTS SURVIVING	NUMBER OF PLANTS PRODUCING ELONGATED AXES	NUMBER OF PLANTS PRODUCING BUDS	NUMBER OF PLANTS PRODUCING FLOWERS
Mass.		31	19	19	19
Fla.		7	5	5	5
F_1		56	50	50	50
F_2	167	93	76	74	73
Md.		27	27	27	27
Fla.		7	5	5	5
F_1		51	47	47	47
F_2	113	103	96	96	95
Bc(F_1×Md.)	83	79	79	79	79

did produce elongated axes, however, all but two formed flower buds and all but four flowered.

The analysis of flowering time which follows has been based upon that portion of each population which actually produced flower primordia and flowers. An assumption has been made that in the two F_2 populations the plants which flowered represent a random sample of the various possible genotypes with respect to flowering behavior, which might have been obtained from the cross; in other words, that there had been no systematic elimination of certain genotypes due to linkage with lethal or unfavorable genes. The author is well aware that this assumption may be incorrect and may have led to serious errors in the calculations, particularly in the case of the Mass.×Fla. cross, where 56 percent of the F_2 population failed to flower for one reason or another; but the procedure followed appears to be the only possible basis upon which an analysis can be made at the present time.

Beginning on August 12, 1943, the vegetative apex and subsequently the developing inflorescence of each plant was examined at two-day intervals until the end of the flowering season. The reproductive condition was scored according to the following 11-graded scale which has been successfully used by TURESSON (1930): plants vegetative, no macroscopic floral primordia −5; macroscopic floral primordia in evidence, but inconspicuous −4; buds small −3; buds larger −2; buds large and swelling (tips of disc florets exposed, corallas of ray florets sometimes open) −1; anthesis of the first disc florets 0; flowering sparse in the inflorescence +1; flowering ample +2; inflorescence in full bloom +3; inflorescence fading +4; entire inflorescence faded +5. From these scores the date of initiation of macroscopic floral primordia, the date of anthesis of the first disc florets in the inflorescence, and the number of days between floral initiation and anthesis could be determined to the nearest day. The data for the various populations are summarized in histograms, figures 2–7, and the means and standard deviations for each population are given.

ANALYSIS OF RESULTS

It has already been pointed out that floral initiation in *S. sempervirens* is under photoperiodic control. Each year the plant produces first, a basal rosette, next, an elongated, vegetative axis, and then, when the proper balance between length of day and night is reached, floral primordia. The data in figures 2 and 3 clearly show the differences between the responses of the three parental strains and of the hybrid populations to the changing photoperiod. These differences are genetic. The small number of late-flowering (Fla.-type) plants obtained in the F_2 generations indicates that the genotype of the Fla. strain must differ from that of the other two strains by at least three gene substitutions with respect to this physiological character.

Floral initiation marks the onset of the reproductive phase of development. This development proceeds at different rates in the various populations, as may be seen in figures 6 and 7, which show the numbers of days elapsing between floral initiation and anthesis for all the individuals in each population. These differences might be due at least in part to the environment provided the rate of flower-bud development were affected by the length of the photoperiod; since the later the initiation of the reproductive process occurs, the shorter is the photoperiod during which bud development takes place. The

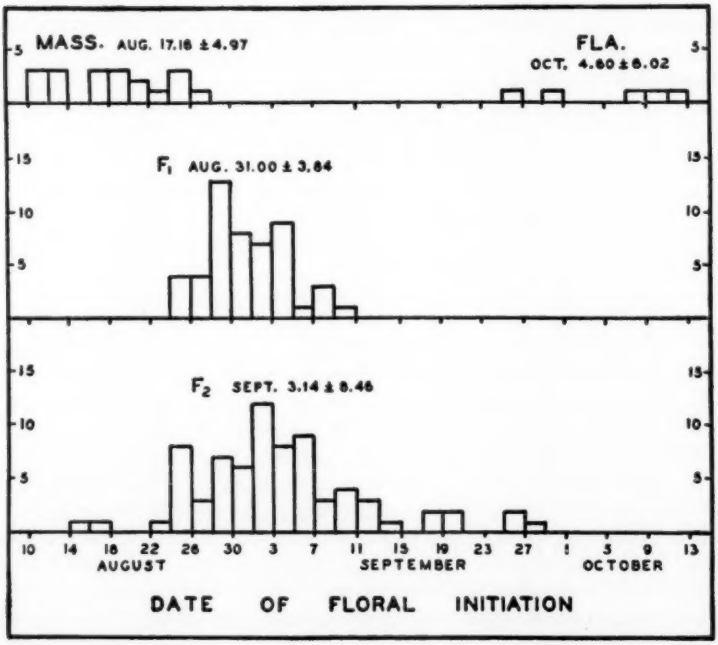
MASS. AUG. 17.18 ± 4.97
FLA.
OCT. 4.60 ± 8.02
F1 AUG. 31.00 ± 3.84
F2 SEPT. 3.14 ± 8.46
10 14 18 22 26 30 3 7 11 15 19 23 27 1 5 9 13
AUGUST
SEPTEMBER
OCTOBER
DATE OF FLORAL INITIATION

FIG. 2

fact that there is a positive correlation among the various populations between mean date of floral initiation for each population and the average number of days for bud development in the population, suggests that the shortening photoperiod may prolong the period of bud development. If this were the case,

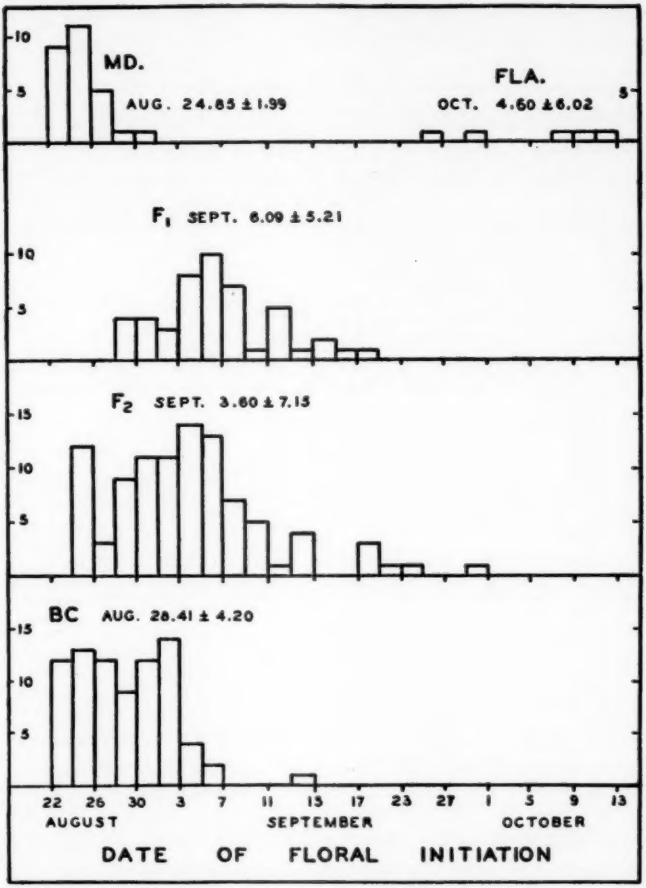

FIG. 3

however, one would expect that there would also be a significant positive correlation among individual plants within a single population between date of floral initiation and rate of bud development. In reality, such correlations are negative rather than positive (see correlations for character pairs 1-2, table 5). It seems probable, therefore, that differences in rate of bud development are determined more by heredity than by the environment.

The actual date of anthesis of the first disc florets in the inflorescence (see fig. 4, 5) will obviously be determined partly by the date of floral initiation and partly by the subsequent rate of development of the flower buds. However, since the date of opening of the first flowers is frequently the observation recorded in studies on flowering time, an analysis of this "character" has been made. It will be shown that this analysis gives an oversimplified picture of the actual genetic situation in these strains of *S. sempervirens*.

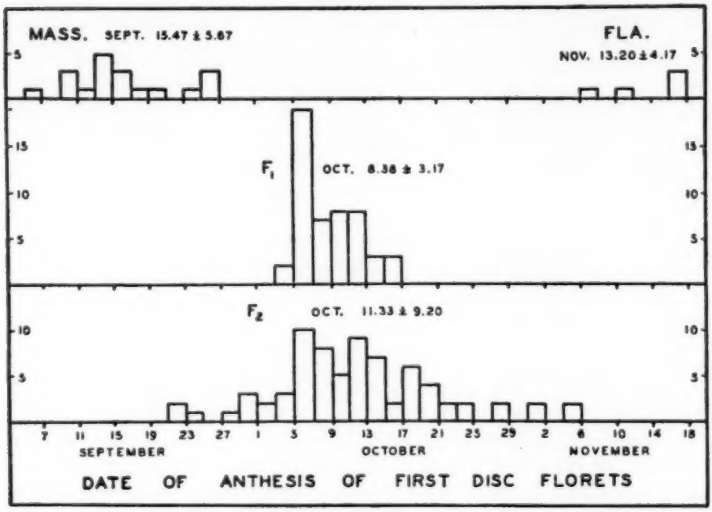
MASS. SEPT. 15.47 ± 5.67
FLA.
NOV. 13.20 ± 4.17
F1
OCT. 8.38 ± 3.17
F2
OCT. 11.33 ± 9.20
7 11 15 19 23 27 1 5 9 13 17 21 25 29 2 6 10 14 18
SEPTEMBER
OCTOBER
NOVEMBER
DATE OF ANTHESIS OF FIRST DISC FLORETS

FIG. 4

Calculation of the number of genes

The number of gene substitutions which determine a difference in some measurable character between two genetic strains may be estimated by a method devised by WRIGHT (in CASTLE 1921), provided numerous individuals of each strain and of their F_1 and F_2 hybrids have been measured with respect to that character. The method is based upon the assumptions that "(1) each strain is isogenic with respect to the character studied, (2) the large-size genes are concentrated in one strain, the small-size genes in the other, (3) the genes involved have equal effects,[2] without dominance or epistasis, and (4) no two of the loci are in the same chromosome. If these assumptions do not match the actual conditions in the material to which the method is applied, the estimated number of gene differences will be smaller than the actual number" (CHARLES and GOODWIN 1943). In any case, a *minimal* estimate will be obtained.

[2] In some cases genes may have non-additive effects which become additive upon proper choice of scale. In such cases the estimate is not minimal unless the most suitable scale has been used. In the present instance no change of scale appears to be required.

The method is embodied in the following formula, in which x_P and x_p represent the average measurements of the parent strains, σ_1 and σ_2 the standard deviations of the F_1 and F_2, respectively, and n the number of gene differences:

$$n = (x_P - x_p)^2/8(\sigma_2^2 - \sigma_1^2).$$

This formula may be rewritten:

$$n = \Delta^2/8(R^2 - 1)$$

where $\Delta = (x_P - x_p)/\sigma_1$, and $R = \sigma_2/\sigma_1$.

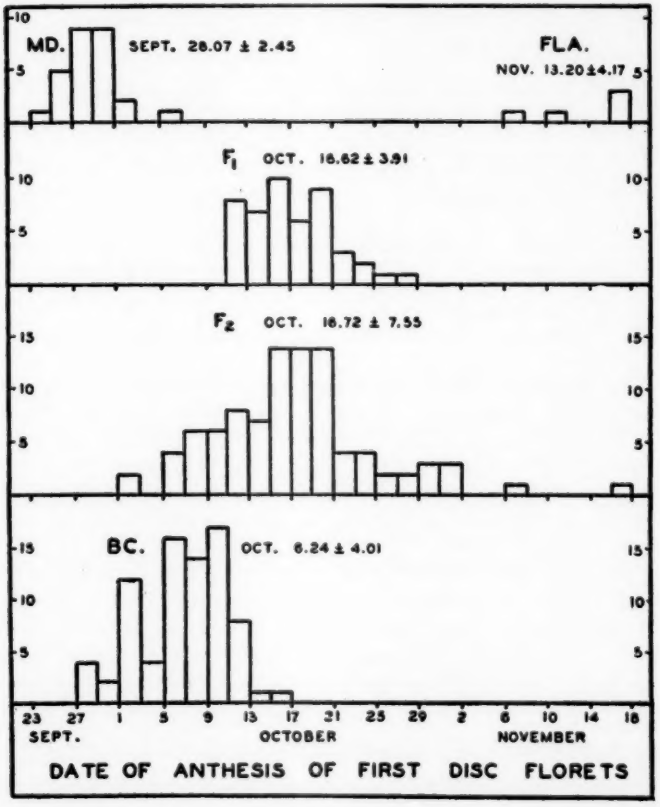

FIG. 5

The significant statistics for differences in date of floral initiation, number of days from floral initiation to anthesis of the first disc florets, and date of anthesis, between the three strains of *S. sempervirens*, and estimates of the number of gene substitutions responsible for these differences, are given in table 4.

TABLE 4

Statistics for the determination of the minimal number of gene substitutions differentiating the Mass. and Fla. and the Md. and Fla. strains with respect to three flowering characters. $n=\Delta^2/8(R^2-1)$. *Exact values for* n *are given in parentheses.*

CROSS	CHARACTER	Δ	R	MINIMAL NUMBER OF GENE DIFFERENCES n
Mass. × Fla.	1. Date of floral initiation	12.61	2.20	6 (5.2)
	2. Number of days from floral initiation to anthesis	5.09	1.51	3 (2.5)
	3. Date of anthesis	18.53	2.90	6 (5.8)
Md. × Fla.	1. Date of floral initiation	7.82	1.37	9 (8.7)
	2. Number of days from floral initiation to anthesis	2.68	1.65	1 (0.5)
	3. Date of anthesis	11.79	1.93	7 (6.4)

Some of the genes influencing the phenotypic expression of one of these three characters might also influence the expression of one or both of the others. A method has been devised to estimate the number of genes with such manifold effects[3] (CHARLES and GOODWIN 1943). The following quantities are used: Δ_x, the difference between parents in average value of character x, divided by the standard deviation of that character in F_1—that is, $\Delta_x=(x_P-x_p)/\sigma_{x1}$; Δ_y, the analogous quotient for character y—that is, $\Delta_y=(y_P-y_p)/\sigma_{y1}$; R_x, the quotient of standard deviations of character x in F_1 and F_2—that is, $R_x=\sigma_{x2}/\sigma_{x1}$; R_y, the analogous character-y quotient—that is, $R_y=\sigma_{y2}/\sigma_{y1}$; r_1, the correlation between characters x and y in F_1; r_2, the correlation in F_2; n_C, the number of genes acting on both characters; n_L, the number of character-x loci each linked with a character-y locus; c, the average crossover value between linked pairs of genes. The method is given by the following formula

$$n_L(1-2c)+n_C=\Delta_x\Delta_y(r_2R_xR_y-r_1)/8(R_x^2-1)(R_y^2-1).$$

The quantities in the right-hand member of the equation are those of table 4, with the exception of the correlation coefficients, which are given in table 5. The correlation coefficients are taken as positive, whatever may be their actual sign. The values for the expression $n_L(1-2c)+n_C$ (the "common gene index") are also given in table 5.

[3] The criticism has been made that the qualifications to this sort of estimate are too numerous to justify the analysis given below. The results, however imperfect, have been presented as an attempt to answer the question, what is the *minimal* complexity of the genetic situation?

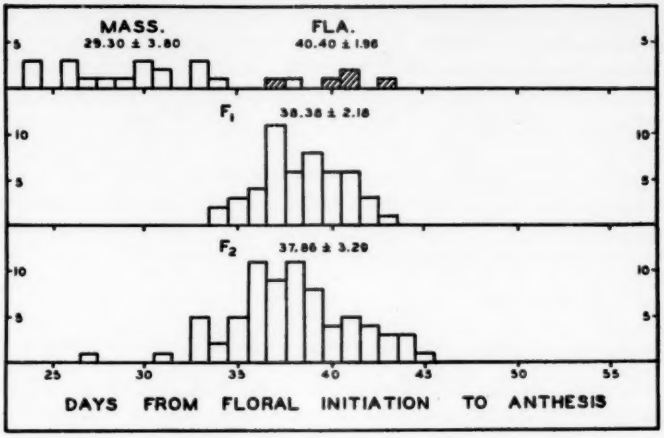
MASS.
29.30 ± 3.80
FLA.
40.40 ± 1.96
F1
38.38 ± 2.18
F2
37.86 ± 3.29
25
30
35
40
45
50
55
DAYS FROM FLORAL INITIATION TO ANTHESIS

FIG. 6

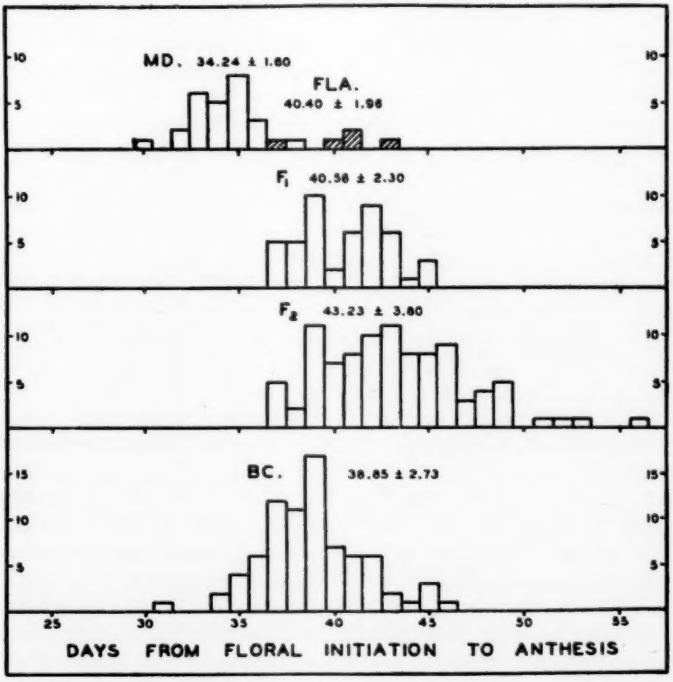
MD. 34.24 ± 1.60
FLA.
40.40 ± 1.96
F1 40.38 ± 2.30
F2 43.23 ± 3.80
BC. 38.85 ± 2.73
25
30
35
40
45
50
55
DAYS FROM FLORAL INITIATION TO ANTHESIS

FIG. 7

TABLE 5

Correlation between the pairs of characters given in table 4 in F_1 and F_2 hybrids between Mass. and Fla. and between Md. and Fla., with an index which is equal to the number of genes acting on both characters plus a function of the number of linked gene pairs (one gene of each pair affecting one character, one the other).

CROSS	CHARACTER PAIR	CORRELATION IN F_1	CORRELATION IN F_2	COMMON GENE INDEX
Mass. × Fla.	1–2	−.563	+.056	−0.6
	1–3	+.823	+.928	5.2
	2–3	+.005	+.408	2.2
Md. × Fla.	1–2	−.726	−.150	−0.7
	1–3	+.916	+.862	6.5
	2–3	−.365	+.370	0.7

The small negative values for this expression obtained between characters 1 and 2 may be interpreted as indicating an absence of linkage and of common genes between the sets of genes determining these characters. Hence, one might postulate a minimum of 6+3=9 gene substitutions differentiating Mass. from Fla., and 9+1=10 gene substitutions differentiating Md. from Fla. Since the haploid chromosome number in *S. sempervirens* is 9 (GOODWIN 1937), at least one instance of linkage would be required in the case of ten gene substitutions.

The maximal estimate for the number of common genes between any particular character pair should be equal to the next integer below the exact value for the expression $n_L(1-2c)+n_C$, since a negative value for linked genes (n_L) would be meaningless. The estimated values for the minimal number of gene differences determining each character difference and the estimated maximal number of genes acting on both members of each character pair are summarized in figure 8. It can be seen that the number of gene substitutions differentiating the strains with respect to "character 3," date of anthesis, estimated directly from the data given in figures 4 and 5, is smaller by three genes than the sum of the estimates for characters 1 and 2, which should, from a developmental point of view, give the more reliable values. This indicates the magnitude of the errors which would have been made in over-simplifying the problem, had the analysis been made upon the date of anthesis alone. The common gene index between date of floral initiation and date of anthesis is large, as might have been expected; and here, it seems safe to assume a maximum value for common genes (n_C), since all the genes affecting the first character must also affect the second.

In conclusion, it would be well to point out certain shortcomings in the above analysis. First, the statistics upon which the estimates depend, and

hence, the estimates themselves, are subject to sampling errors due to the small size of the populations measured. Second, the estimation method is based upon the assumption that the crosses are between isogenic strains. The P_1 plants in the present instance were grown from seeds of wild plants or were only one generation removed from wild seeds, and there is some suggestion of genetic heterogeneity at least among individuals of the Mass. strain. Third, the possibility of a systematic elimination of certain genotypes, particularly in the Mass.×Fla. cross, has already been mentioned. Fourth, no correction has been made for dominance of the genes influencing the rate of bud development. And last, the estimations are aimed at mininal, rather than the most probable

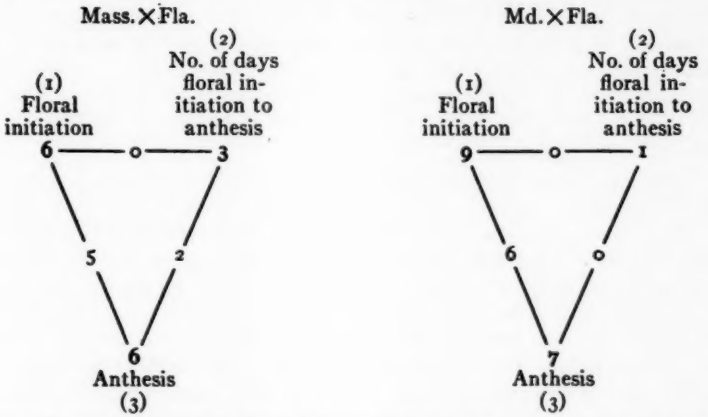

FIG. 8.—Flowering characters differentiating three strains of *S. sempervirens*, estimated minimal number of gene differences determining each character difference (bold-face digits at apices of triangles), and maximal number of genes acting on both members of each character pair (digits on lines connecting the characters).

values. In spite of these difficulties, the results have been presented in the hope that even this somewhat crude analysis will prove interesting and instructive.

DISCUSSION

The recent investigations of CAJLAHJAN (1936), MELCHERS (1936), KUIJPER and WIERSUM (1936), HAMNER and BONNER (1938), MELCHERS and LANG (1941), and others indicate that the flowering of various short-day plants is controlled by one or more flowering hormones which are produced in the leaves under suitable photoperiodic conditions. It might be inferred that genetic strains differ with respect to the conditions under which these hormones are produced or transported within the plant. Since no experiments have as yet been carried out to determine the mechanism of photoperiodic control of flowering time in *S. sempervirens*, this interesting subject is largely a matter of speculation.

The results of this investigation serve to emphasize once again the com-

plexity of the problem of natural speciation. In *S. sempervirens*, gene substitutions responsible for differentiating strains with respect to their flowering behavior are numerous, probably occurring in every chromosome. Physiological characters of this sort are doubtless of great importance in limiting the distribution of a species (see ALLARD 1932, 1943).

It would be of interest to know something about the number of flowering-time genes differentiating strains of some of the other species which have been investigated. Unfortunately, most of the papers thus far published have not included data taken on simultaneously-grown P_1, F_1 and F_2 populations; but such data for P_1 and F_2 populations are sometimes given. In WRIGHT's formula, which is based on the assumption that the P_1 strains are isogenic with respect to the character studied, the variance of the F_1 is used as a measure of the variability of individuals of the same genotype due to the environment. If the variance of the P_1 populations, $(\sigma_P^2+\sigma_p^2)/2$, or $(N_P\sigma_P^2+N_p\sigma_p^2)/(N_P+N_p)$, where N_P and N_p are the numbers of individuals in the P_1 populations, is substituted for the variance of the F_1, the method can then be applied to data already in the literature.

Table 6 gives the results of estimates on three different genera of plants. In the intervarietal cotton cross (LEAKE 1911) the minimal estimate is slightly larger than the haploid chromosome number, which suggests that here, as in the strains of *S. sempervirens*, there is at least one flowering-time gene in each linkage group. In a cross between two strains of rice, an estimate of four genes is in fairly close agreement with the three factors previously hypothesized (HOSHINO 1915). In various wheat crosses (THOMPSON 1918; FREEMAN 1919; FLORELL 1924) the estimated number of genes ranges between one and five. KUCKUCK (1930) has postulated three different genes to account for the flowering behavior of F_2 and F_3 populations in certain barley crosses, a value comparable to those given in table 6 for the other grains.

It would also be instructive to check WRIGHT's method against a known situation. RASMUSSON (1935) has reported the results of a series of crosses between six different strains of peas, in which at least two genes partially dominant for lateness in flowering time are involved. He claims that these two genes account for about one-half of the observed differences in flowering time between the various strains. In table 7 a comparison is made between the number of genes known to be involved in these crosses and the minimal number estimated by WRIGHT's method. In all crosses but one, the number estimated is equal to, or slightly smaller than, the value given by RASMUSSON's analysis. In the *WW*×*Gj* cross, the smaller value obtained is to be expected, since the genes for lateness are divided between the parents, a condition which is contrary to one of the basic assumptions made in the derivation of the formula. The *Bism*×*Gd* cross gives an estimated value of four genes, which suggests that there are at least two genes in operation in addition to the two described by RASMUSSON, a result entirely compatible with his conclusions. No data were given as to the size of the populations, and errors in the estimates may have been made if the populations from which the variances were computed were

TABLE 6

Estimates of the minimal number of genes differentiating strains of cotton, rice, and wheat (exact values given in parentheses).

CROSS	$X_P - X_p$	$\frac{N_P\sigma_P^2 + N_p\sigma_p^2}{N_P + N_p}$	σ_z^2	NUMBER OF GENES ESTIMATED	AUTHOR
Gossypium arboreum L. (n=13)×*G. arboreum* var. *assamica* Watt. (type 10) (n=13)	51.60	47.30	71.99	14 (13.5)	LEAKE (1911)
Oryza sativa L. (n=12) Kuro-Bozu×Akage	34.70	2.87	50.63	4 (3.2)	HOSHINO (1915)
Triticum					
Red Fife×Club	5.53	2.68	8.77	1 (0.6)	
Prelude×Bobs	5.90	1.49	5.70	1 (1.0)	
Bobs×Preston	8.64	2.03	9.46	2 (1.3)	
Marquis×Alaska	8.85	2.33	20.09	1 (0.6)	
Prelude×Marquis	12.42	2.06	7.78	4 (3.4)	THOMPSON
Prelude×Preston	14.54	2.07	12.70	3 (2.5)	(1918)
Bobs×Alaska	15.37	2.09	28.81	2 (1.1)	
Bobs×Club	16.02	2.13	16.08	3 (2.3)	
Prelude×Red Fife	16.39	2.31	10.34	5 (4.2)	
Prelude×Kubanka	21.10	2.26	29.94	2 (2.0)	
Algerian macaroni (#1) ×Sonora (#35)	14.00	9.63	16.00	4 (3.8)	FREEMAN (1919)
Algerian red bread (#2) ×Early Baart (#34)	12.00	2.90	15.84	2 (1.4)	FREEMAN (1919)
Sunset×Marquis	19.22	6.09	28.00	3 (2.1)	FLORELL (1924)

very small. It should be pointed out further that no correction[4] has been made for the partial dominance of the two genes which have been analyzed by RASMUSSON.

SUMMARY

A study has been made of the inheritance of flowering time in the short-day species, *Solidago sempervirens*. Three strains, originally isolated from natural populations, could be distinguished from one another both by their dates of

[4] A correction for dominance, which will always tend to increase the estimate, may be made by adding the expression $8(x_{F_1} - x_{F_2})^2$ to the numerator of WRIGHT's formula, where x_{F_1} and x_{F_2} represent the average of measurements of the F_1 and F_2, respectively. Unfortunately, the necessary data are not available in RASMUSSON's paper. If complete dominance is assumed, an estimate may be obtained by multiplying WRIGHT's formula by 1.5 (SEREBROVSKY 1928). Such a correction might possibly increase the estimates for the *Bism*×*Gj* and *Bism*×*Gd* crosses by as much as one gene, but the others would not be affected.

TABLE 7

A comparison of the number of genes known to differentiate certain strains of peas and the number estimated by Wright's method. Data are from Rasmusson (1935).

CROSS	P_1 GENOTYPE*	THE NUMBER OF GENES: KNOWN TO DIFFERENTIATE STRAINS	THE NUMBER OF GENES: ESTIMATED BY WRIGHT'S METHOD	$X_P - X_p$	$\frac{\sigma_P^2 + \sigma_p^2}{2}$	σ_2^2
Gj×EsII	*aaBB×aaBB*	0	0	0.2	5.10	7.98
St×Gd	*aaBB×AABB*	1	0	0.4	13.04	7.43
WW×Gd	*AAbb×AABB*	1	0	5.6	8.83	7.74
Bism×Gj	*aabb×aaBB*	1	1	10.6	5.82	19.92
WW×Gj	*AAbb×aaBB*	2	1	7.4	4.60	18.56
Bism×Gd	*aabb×AABB*	2	4	9.1	10.06	13.26

* *A* represents the gene *le*; *B* the gene X_a. Both of these genes are partially dominant for retardation of flowering.

floral initiation, which are controlled primarily by the length of the photoperiod, and by their rates of bud development. From an analysis of F_1 and F_2 hybrid populations, it has been estimated that the minimal number of gene substitutions which must determine the above-mentioned physiological differences between these strains approximates the haploid number of chromosomes in the species (n = 9); and it is probable that these genes are located in many, if not all, of the linkage groups.

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ISO-ALLELES AT THE BOBBED LOCUS IN DROSOPHILA HYDEI POPULATIONS

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THE mutant, bobbed, is among those which have been recorded frequently in the genus Drosophila. It was first found by BRIDGES in *Drosophila melanogaster* in 1915, and 40 mutations at this locus have been listed for the species. Of these, half were found by BRIDGES (BRIDGES-BREHME 1944). Parallel mutations have been recorded in *D. simulans* (STURTEVANT 1929); in *pseudoobscura* (STURTEVANT and TAN 1937); in *affinis* (STURTEVANT 1940); in *ananassae* (MORIWAKI 1935; KIKKAWA 1938); in *subobscura* (JERMYN, PHILIP, RENDEL, and SPURWAY 1943); in *hydei* (CLAUSEN 1923; SPENCER 1927); in *funebris* (TIMOFÉEFF-RESSOVSKY 1931; SPENCER 1934); and in *virilis* (CHINO 1936). In all of these species except *ananassae* the locus of bobbed occupies a position at the centromere end of element A. This places it in a terminal position on the genetic map of the X chromosome when this contains only one euchromatic arm or in a central position on the map where there are two euchromatic arms in the X as in *pseudoobscura* and *affinis*. In *D. ananassae* KIKKAWA (1938) has shown that the locus is in the small fourth chromosome. In general the mutant is strongly sex-limited in phenotypic expression. Through studies on non-disjunction and gynandromorphs STERN (1926a, 1927) first showed that the Y chromosome carried a bobbed locus, and thus accounted for phenotypic differences in male and female when X and Y chromosomes carried different alleles. Later, by combining cytological and genetic techniques, STERN (1929a) demonstrated crossing over between the X and Y chromosomes. By using females varying cytologically from XX to XXYY and males varying from X to XYY in constitution, and carrying different combinations of bobbed and non-bobbed alleles, including a bobbed-lethal, he was able to demonstrate the additive effects of two or more genes in conditioning bristle size (STERN 1929b).

The bobbed phenotype includes a reduction in the size of all bristles, abnormal abdominal sclerites, lengthening of larval-pupal period, lowered fecundity, fertility and viability, and lethal effects in homozygotes in extreme alleles. In species other than *D. melanogaster* the most extensive analysis and account of the bobbed phenotype has been given by LÜERS (1937) for a dominant bobbed allele in *D. funebris*. In *D. simulans*, STURTEVANT (1929) has shown that Y chromosomes derived from stocks collected from widely separated geographical areas carried bobbed alleles more extreme than the three mutations to bobbed in the X found in different laboratory stocks. Consequently males showed a more extreme bobbed phenotype than females. One wild Y chromosome derived from a Woods Hole stock, however, when combined

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with a mutant X gave males no more extreme than the females of the mutant stock. In *D. pseudoobscura*, STURTEVANT and TAN (1937) have found a condition similar to that in *D. simulans*.

Bobbed was first discovered and reported in *D. hydei* by CLAUSEN (1923). The allele found by him produced a moderate reduction in bristle size and some abnormality in abdominal sclerites. In November, 1925, the author collected a few rotting tomatoes, in which Drosophila were breeding, from a fruit cellar in Wooster, Ohio. *D. hydei* derived from this collection was bred in mass culture until March, 1926, when 50 pair matings were made up. Three of these gave some females with reduced bristles, and from one of them by further inbreeding a pure bobbed stock was isolated. Compared to CLAUSEN'S bobbed this stock was more extreme in bristle reduction but without abnormal abdominal sclerites. During subsequent work on this species mutations at other loci were found and laboratory stocks built up from collections made at different times. It was noticed from time to time that some laboratory strains contained bobbed and it was assumed that these had been contaminated either accidentally or in experimental crosses from CLAUSEN'S bobbed, of which a stock had been secured, and from the original Wooster bobbed stock. The facts to be presented in this paper, however, would indicate that bobbed was probably present in the original wild strains from which the laboratory stocks were derived. A brief summary of the data on bobbed alleles in *D. hydei* has been reported elsewhere (SPENCER 1938).

THE AZUSA POPULATION OF DROSOPHILA HYDEI

In the winter of 1936–1937 the author, then at the CALIFORNIA INSTITUTE OF TECHNOLOGY, received from DR. J. SCHULTZ a small sample of *D. hydei*, collected at a citrus dump near Azusa, Southern California. These flies were mated in pairs, and from them several new strains of bobbed were isolated, differing among themselves and from the original alleles. Certain other mutants appeared in the material, and it was decided to make an extensive study of this population.

The citrus dump was located about a mile from Azusa and thirteen miles from Pasadena in a tract of waste land near the San Gabriel River. Here were many tons of rotting citrus fruit, mostly oranges, and casual inspection showed that the citrus dump supported a huge population of *D. hydei*. The adults were there by the million, and the material contained great masses of larvae. Pupae were collected by scooping them up with a trowel. During the spring when the population was at its peak Drosophila could be trapped in almost incredible numbers at a considerable distance from the breeding ground. One half-pint bottle containing fermenting banana mash was hung in a bush about fifty yards from the citrus dump on April 21 and taken in at 4:30 P. M. on April 23, temperature 23°C. It contained 1403 Drosophila, of which 846 were *D. hydei* males and 357 *D. hydei* females. On March 20 a collection was made by holding a cellophane sack over crevices in the mass of citrus fruit. In a few minutes 933 males and 321 females of *D. hydei* were taken along with 14 *D. immigrans*, three D. *pseudoobscura*, two *D. melanogaster*, and one *D. busckii*. Both of these

collections were made in cool weather, and the high preponderance of males was due to the fact that in this species the males are more active than the females at low temperature and not to any such abnormal sex ratio as the collection figures alone would indicate.

It was therefore decided that a truer sample of the population could be secured by making collections of mature pupae and rearing them out. The pupae were collected in large open cans which were placed in paper sacks. These sacks were pinned shut, and daily collections of the adult flies emerging were made from them. These adults were then taken to the laboratory and examined under the binocular microscope for mutant characters.

BOBBED PHENOTYPES IN THE AZUSA POPULATION

Examination of these flies showed that a considerable number of the females were phenotypically bobbed; these ranged from extreme types in which the bristles were reduced to the size of large hairs to slightly reduced bristle forms. It soon became apparent that the less extreme bristle types graded up close to or into the normal bristle form. The normal type could easily be checked, since all males belonged to this class. The flies were quite uniform in size, indicating optimum food conditions; no difficulty in classification due to undersize flies was encountered.

Of the 27,805 flies examined there were *D. hydei* males, 12,031, females, 15,575; *D. melanogaster* males, 45, females, 62; *D. immigrans* males, 32, females, 40; *D. pseudoobscura* males, one, females, nine; *D. busckii* males, five, females, five. Of the 15,575 *D. hydei* females 495 or 3.1 percent were classified as phenotypically bobbed. Only those flies showing distinct bristle reduction were so classified, and it was apparent that many slight bobbed individuals were being placed in the normal class. The significantly lower number of males was probably due to the presence of some sex-linked lethals in the wild population, and more particularly to sex-linked recessive semi-lethal and lowered viability mutants. Large scale laboratory experiments on Drosophila have shown similar sex-ratios.

One vermilion, one extreme scute, and one light male, the first two sex-linked recessives and the latter a sex-linked semi-dominant, were found. These were of interest in connection with the report of DUBININ and collaborators (1937) that sex-linked recessives were practically absent from very large collections of *D. melanogaster*. Among 129,582 flies taken they found only one sex-linked mutant, a yellow male. However, more recently BERG (1942a, 1942b) has collected large numbers of sex-linked mutant males, mostly yellow, in certain populations of *D. melanogaster*. STURTEVANT (1915) has reported a considerable number of the mutant, light, a sex-linked semi-dominant, in collections of *D. repleta*. METZ found a yellow male in a collection of *D. simulans* (STURTEVANT 1929). The author has found yellow in wild specimens of both *D. melanogaster* and *D. immigrans* and has recorded vermilion in large numbers in the Wooster population of *D. hydei* (SPENCER 1932). While selection undoubtedly tends to reduce the numbers of sex-linked mutant individuals

the supposition that they are almost non-existent in wild populations would seem to be erroneous, at least under certain breeding conditions.

BOBBED GENOTYPES IN THE AZUSA POPULATION

The presence of a complex series of bobbed phenotypes in the Azusa population could have been due either to one bobbed allele acted on by modifiers or to multiple alleles or to a combination of these causes. Tests were set up as follows. Since the females reared from pupae were virgin when collected (minimum age of sexual maturity after eclosion in this species is two days for the females and five days for the males), phenotypically bobbed females of different grades were mated singly to standard bobbed males. Some tests were run using the Wooster bobbed stock, others using the bobbed stock of CLAUSEN, hereafter designated as Berkeley bobbed. The females chosen for these tests constituted a representative sample taken from the 495 bobbed females collected from the wild population, grading from slight to extreme types. In all, 54 such females were tested. When their offspring emerged, the daughters fell into either one or two classes with relatively little variation among the members of a class. Five members of each class were measured with an ocular micrometer for length of posterior scutellar bristles.

These data are shown graphically in figure 1, with full explanation in the legend to this figure. It will be noted that all but 15 of the females tested contained in their two X chromosomes separable bobbed alleles which in heterozygous combination with the tester bobbed allele gave two distinct phenotypes in the daughter offspring. The distribution of the daughter phenotypes, either one or two grades in each test, but many grades when all the tests were considered, indicated that the phenotypic variations were not due to recessive genetic modifiers acting on one bobbed allele, for in heterozygous condition the effect of such modifiers would be lost. There was, of course, some variation in bristle length in individuals owing to size differences in the flies, and it is likely that separable alleles could have been demonstrated in some of the 15 females referred to above by extensive tests and statistical analysis. The heterozygous daughter classes ranged from bristle grade 12, posterior scutellar bristle length 252 microns, to grade 33, posterior scutellar bristle length 693 microns, the standard bristle grade of males.

In all, 40 backcross tests of heterozygous daughter classes carrying one Wooster bobbed tester gene and one wild Azusa gene to Wooster bobbed males were set up. These included daughters from 16 of the original tested females and of the following grades: 12, 13, 14, 17, 18, 19, 22, 24, 25, 27, 28, and 29. However, none of the tests for grades 12, 13, or 14 were successful, although 18 cultures were started. The extreme bristle grades are generally highly infertile. At least some cultures of all the other grades tested came through, and the results were uniformly consistent with the interpretation that the bobbed variations were due to alleles at that locus rather than to dominant modifiers. In table 1 data are presented of the micrometer measurements made on posterior scutellar bristles of males and females of Wooster

bobbed stock and from several of these backcross experiments. The data show the usual range of variability in bristle length within the members of each class in the backcross tests. It will be noted that the variability in the females of a class is no greater than the variability in the males from the same culture, or in males and females from the inbred tester stock. Minor variations in the

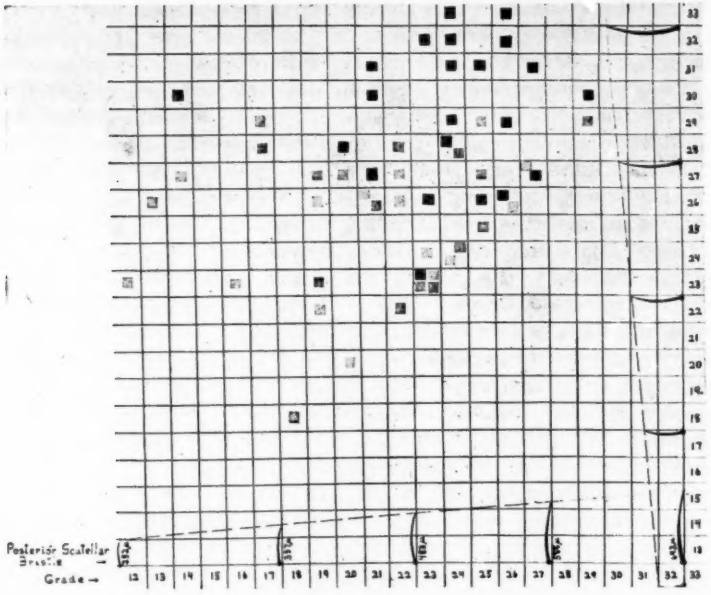

FIG. 1.—Tests of 54 phenotypically bobbed females reared from pupae collected from the Azusa population of *Drosophila hydei*. The position of each square designates the bristle length or grade in the heterozygous daughters from the mating of an Azusa bobbed female to a male from a standard bobbed stock. A black square indicates mating to a Berkeley male, the X chromosome of which carried a bobbed gene which in XX females of the stock gave a bristle grade of 28, slight bobbed. A gray square indicates mating to a Wooster male carrying a grade 20, medium bobbed, gene in the X chromosome. For example the square at the extreme left of the figure indicates a mating of a female from the Azusa population to a Wooster male carrying grade 20 bobbed in his X chromosome. The female carried in one X a gene which in heterozygous combination with the grade 20 tester gave daughters of grade 12 and in the other X a gene which in heterozygous combination with the grade 20 tester gave daughters of grade 23. It will be noted that all but 15 of the females tested carried two alleles distinguishable in the heterozygous combinations with the tester gene used.

mean bristle length of males in different cultures was due to environmental variables difficult to control. These environmental factors would likewise be expected to produce such variation within a class of females as was observed. By referring to figure 1, it will be noted that in two cases where the Berkeley tester was used in analysing bobbed females one class of daughters was pheno-

typically non-bobbed. In general it will be noted that the heterozygous combinations with Berkeley grade 28 gave an average bristle length greater than the combinations with the more extreme Wooster grade 20 tester. It was obvious that the complex series of bobbed alleles in heterozygous combinations showed no dominance within the series, but rather gave phenotypic classes conditioned by the strength of the two alleles present in the heterozygote.

The test of the female represented by the square at the extreme left of figure 1 was of particular interest. One class of her daughters was of grade 23, the other of grade 12. She was the most extreme bobbed type recovered from the wild population. It seemed likely that if these grade 12 daughters, carrying the Wooster bobbed 20 gene, showed an extreme phenotype of greatly reduced bristles and abnormal abdomen, that the wild gene recovered from their mother would be lethal in homozygous form. To test this a series of pair matings of these daughters to their brothers, all phenotypically alike but half carrying the extreme, presumably lethal, wild gene, and the other half the less extreme wild gene, was made up. Some of these matings gave two classes of daughters, one of grade 23 and the other of less extreme grade. The other matings produced only one type of female, grade 12, and the sex-ratio indicated the presence of a bobbed-lethal, killing off half of the females.

TABLE 1

Frequency distributions of males and females from Wooster bobbed stock and of the classes of males and females from four of the backcross tests of heterozygous wild Azusa/Wooster bobbed females to Wooster bobbed males.

GRADE OF POSTERIOR SCUTELLAR BRISTLES	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Wooster bobbed^{20} males													1	1	4	19	2	1
Wooster bobbed^{20} females				5	4	4												
Sons of $(Wb^{20}/Az)^{16}$ female × Wb^{20} male															2	5	6	7
daughters of $(Wb^{20}/Az)^{16}$ female × Wb^{20} male		2	11	10	2	4	2	2										
Sons of $(Wb^{20}/Az)^{23}$ female × Wb^{20} male																1	7	2
Wb^{20}/Wb^{20} daughters of $(Wb^{20}/Az)^{23}$ female × Wb^{20} male		1	2	3	2													
Wb^{20}/Az daughters of $(Wb^{20}/Az)^{23}$ female × Wb^{20} male								1		5	5	2						
Sons of $(Wb^{20}/Az)^{28}$ female × Wb^{20} male														2	2	6	6	
Wb^{20}/Wb^{20} daughters of $(Wb^{20}/Az)^{28}$ female × Wb^{20} male	1		1	3														
Wb^{20}/Az daughters of $(Wb^{20}/Az)^{28}$ female × Wb^{20} male											1	3	2	8	3			
Sons of $(Wb^{20}/Az)^{29}$ female × Wb^{20} male															2	5	1	
Wb^{20}/Wb^{20} daughters of $(Wb^{20}/Az)^{29}$ female × Wb^{20} male			3	3	2		1											
Wb^{20}/Az daughters of $(Wb^{20}/Az)^{29}$ female × Wb^{20} male												3		2	2	1		

While the above analysis has demonstrated the presence of a large series of multiple alleles in the Azusa population, through the testing of phenotypically bobbed wild females, it also showed that the mere tabulation of wild females as bobbed or non-bobbed could not give an accurate quantitative picture of the frequencies of the many bobbed grades present in the genotypes. Since all males seen in the population were phenotypically alike, grade 33, there was no chance of error through selection of a weighted sample in the following test (Note: the male recorded as Y-bobbed in Drosophila Information Service No.

11, page 17, was later shown by linkage tests not to be bobbed). One hundred and six Azusa wild males were pair-mated to standard grade 20 Wooster bobbed females. The results of this test are shown in figure 2. While the sample may be too small to be entirely representative, it should furnish an approximate analysis. It will be seen that of the 106 genes tested, 27, or 25.5 percent, gave phenotypically bobbed daughters. Each test gave daughters of a single grade. These ranged from an extreme of grade 17 to very slight of grade 32. Further tests of representatives from the heterozygote grades 27 to 32 showed that when these genes were homozygous, they had no visible bobbed effects. They had been distinguished from one another only by the use of the fairly

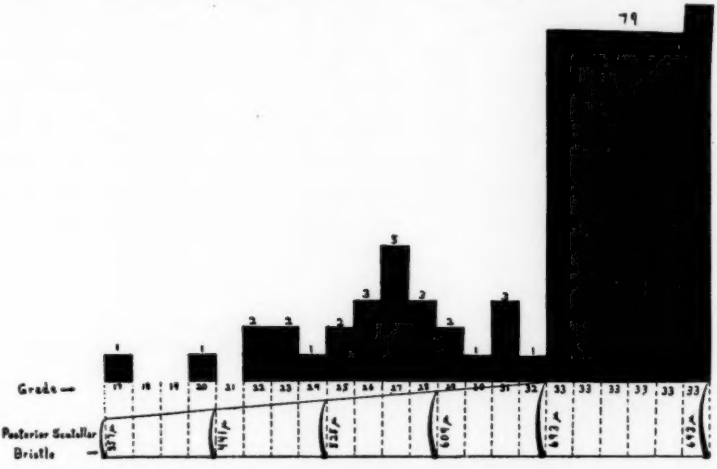

FIG. 2.—Bristle length in the heterozygous daughters of 106 Azusa males, all phenotypically identical, mated to Wooster grade 20 females. Twenty-seven or 25.5 percent of males carried alleles in their X chromosomes forming phenotypically bobbed compounds with grade 20 tester genes.

strong Wooster bobbed 20 as a tool. Following STERN and SCHAEFFER (1943), we shall refer to these and other genes in the series, requiring testers to distinguish them and to seriate them, as iso-alleles. Such iso-alleles are indistinguishable from one another in homozygous form.

Since Wooster bobbed 20 had given more extreme heterozygous combinations than Berkeley bobbed 28, it was assumed that the bobbed-lethal extracted from the Azusa population would prove a still more effective tool. It was therefore made up in heterozygous combination with a grade 22 bobbed also extracted from the Azusa population. This combination was somewhat more viable and fertile than Wooster 20/Azusa lethal. The Azusa 22/Azusa lethal females were pair-mated to 12 wild Azusa males collected from the population and all phenotypically alike. The results of these matings are shown in figure 3. It will be noted that of the 12 wild genes present in the 12 Azusa males picked at random three of them, designated b^a, b^b, and b^c, gave phenotypically

bobbed heterozygotes with Azusa 22 and much more extreme heterozygous combinations with Azusa lethal. In fact b^a gave a lethal combination with the latter and probably was itself a homozygous lethal, although the test was not made. Furthermore, of the nine genes which gave phenotypically non-bobbed heterozygotes with Azusa 22, three designated as b^d, b^e, and b^f, gave phenotypically bobbed combinations but of visibly different grades with Azusa lethal.

Thus it was shown that certain bobbed genes, which not only in homozygous condition but even when acting in heterozygous combination with the rather strong Azusa 22 gave no phenotpyically bobbed effects, could be seriated as to their bobbed potency by the use of the strong bobbed-lethal tester. How-

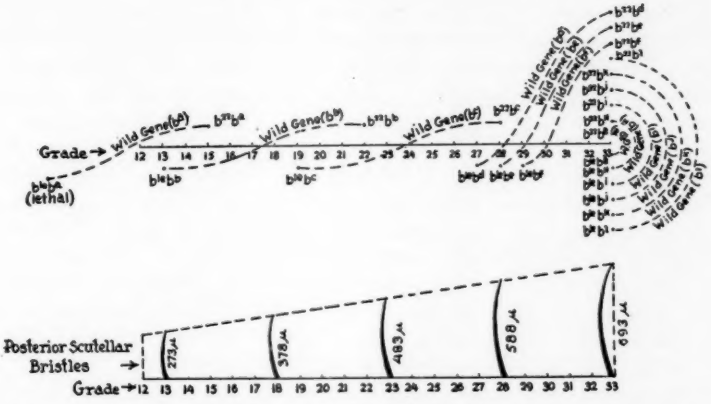

FIG. 3.—Tests of 12 Azusa males to heterozygous tester females of composition Azusa bobbed 22/Azusa bobbed-lethal. Genotypes of each daughter class placed directly above their corresponding phenotypes, represented in the reference scale of bristle lengths. Grade 33 (693 microns) is maximum bristle size in the species. The bobbed-lethal tester distinguishes and seriates six of the genes tested; bobbed 22 distinguishes and seriates three of them.

ever, other genes were so strong in their bristle forming potency as to pull the heterozygotes with the extreme bobbed-lethal into the class of maximum bristle size.

In this presentation of data we have purposely avoided the use of the term wild type because it connotes a homogeneous class of genes in the wild population of which the mutant form is the rare exception. Actually the situation at the bobbed locus in the Azusa population has been shown by this analysis to be quite different. There is no one wild type allele mutating rarely to bobbed. Rather there is a complex series of bobbed alleles grading from extreme lethal types at one end and through genes which in homozygous condition give bristles of many different grades to those at the other end, which in homozygous condition are non-bobbed phenotypes but in heterozygous combination with moderate bobbed genes show visible effects. Finally at this end of the series there are some genes so strong in bristle forming potency as to override

the effect of bobbed-lethals in heterozygous combinations. This, however, does not prove that these form a homogeneous class. By implication they do not. It would be of interest to test for iso-alleles in the more extreme members of the series by using such a gene as b^g (see fig. 3) as a tool in searching for more extreme bobbed-lethals, and using such a lethal in turn in locating and seriating extremes at the other end.

While bristle grade 33 represents the end point in a morphological series, the evidence indicates that minimum grade 33 is far from the end point in what may be designated as a potency series. Using the morphological guide of bristle length in that range of the series where phenotypic effects are secured in heterozygous combinations, we might assign to gene b^c a potency of about 34, b^d a potency of 50, b^e of 52, and b^f of 54. At the other end Azusa lethal would have a potency of about four, and b^a of about eight, itself a homozygous lethal but less extreme than the Azusa lethal tester gene. Heterozygous or homozygous females in which the sum of the potencies of the two alleles present is 66 or more are of maximum bristle grade 33. A potency series was set up by STERN (1929b) to represent the additive effects of bobbed alleles in *D. melanogaster* in haplo, diplo, triplo, and even more complex combinations. A series of this nature was postulated by MOHR (1932) to explain the action of different alleles at the vestigial locus in *D. melanogaster*. It should be mentioned that the effects of some bobbed alleles found in *D. melanogaster* cannot be interpreted in terms of a simple potency series such as proposed here. Such exceptions can better be explained by the substrate-gene interaction theory recently developed by STERN (1943). It seems probable that an extended study of the moderate *D. hydei* bobbed alleles in primary nondisjunction males and in heterozygous combinations with bobbed-lethals would show similar exceptions best interpreted by the substrate-gene interaction theory.

BOBBED PHENOTYPES IN THE WOOSTER POPULATION

In order to determine whether the presence of multiple alleles at the bobbed locus was a characteristic of the species, *D. hydei*, or peculiar to certain populations such as that at Azusa, an investigation of a population of *D. hydei* in the environs of Wooster, Ohio, was made in the fall of 1937 and the summer of 1938. This population was breeding on a large refuse dump maintained back of a wholesale fruit and grocery concern. Here were thrown banana stalks, over-ripe bananas, citrus fruit, melons, tomatoes, and other vegetables. At the peak reached in this latitude in October and November the adult population was estimated at not less than 500,000, much smaller than the Azusa population, estimated at 100,000,000.

The year round structure of these two populations differed greatly as reported by the author (SPENCER 1938, 1941). Since *D. hydei* is native to the tropics, the Wooster population was greatly reduced in the winter, where all survivors overwinter in buildings. In a collection of flies reared from pupae taken from this population in July 1938 of a total of 2,251 there were 813 *D. hydei* males and 1,422 *D. hydei* females. Of these latter 51 or about 3.6 percent

were classified as distinctly bobbed, of which the most extreme was grade 16. Another collection yielded a total of 2,083 flies, 11 *D. melanogaster*, three *D. busckii*, 891 *D. hydei* males, and 1,178 *D. hydei* females. A careful check of the latter showed 57 or 4.8 percent visibly bobbed. As in the Azusa population, several wild males showed other sex-linked recessives, seven vermilion and one singed. In other collections, in which bobbed was not recorded, of 1,220 males 12 were vermilion, one extreme scute and eight light, a sex-linked semi-dominant body color.

BOBBED GENOTYPES IN THE WOOSTER POPULATION

In November, 1937, a test was made to determine the distribution of bobbed alleles in the Wooster population in comparison to that in the Azusa population. In order to secure maximum information for the work involved,

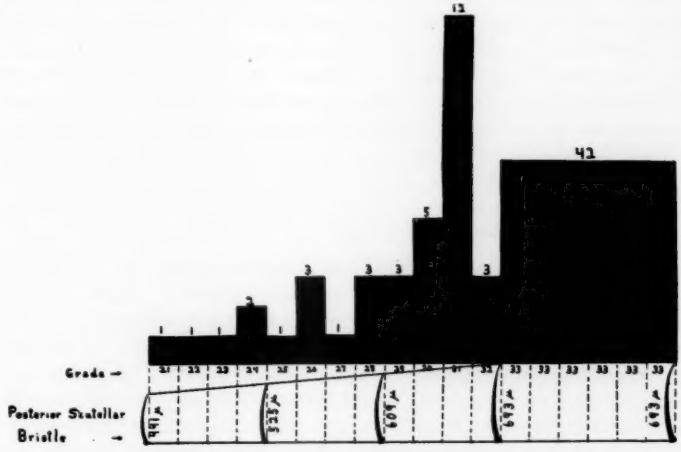

FIG. 4.—Tests of 78 genes carried in 39 females chosen at random from a Wooster, Ohio, population of *Drosophila hydei*. The graph shows bristle lengths in the heterozygous daughter classes from matings of the 39 females to males carrying Wooster grade 20 tester gene in their X chromosomes. Thirty-six or 46.2 percent of genes tested formed phenotypically bobbed compounds with the grade 20 tester.

virgin wild females were pair-mated to Wooster bobbed 20 males. Two genes were tested by each pair-mating. Care was taken that this sample should not be weighted either by selecting or excluding phenotypically bobbed females for the test. The results are shown in figure 4. Of the 78 genes tested 36, or 46.2 percent, formed phenotypically bobbed compounds with standard bobbed 20. This is in contrast to the 25.5 percent of genes of these grades in the Azusa sample. However, the sample shows relatively more of the heterozygotes from the Wooster population in the high grade ranges, 30–32. This might well account for the smaller difference in the percentages of visibly bobbed females in the two populations (3.1 percent for Azusa versus 3.6 to 4.8 per-

cent for Wooster) than might be expected if only the quantity and not the quality of the alleles demonstrated in the two populations were considered. The frequencies of the bobbed alleles in the two populations falls into line with the analysis of gene frequencies at other loci which the author has reported on briefly (SPENCER 1938, 1941). The Wooster population has shown a higher concentration of a few mutant genes, gray body color, vermilion eye, nicked wing, but a lower number of mutant loci per 100 flies tested than were present in the Azusa population. The year-round pattern of the Wooster population gives a sharper bottle-neck effect due to winter killing. In like manner the Wooster population shows a higher concentration of a few bobbed alleles and less spread in the grade range.

OTHER DROSOPHILA HYDEI POPULATIONS

While no quantitative data have been secured from other *D. hydei* populations, it should be noted that small collections from Coffeyville, Kansas, and Long Island have yielded bobbed. In analysing about 100 males from Gatlinburg, Tennessee, for the presence of mutants at other loci and with no intention of checking for bobbed, a lethal at the bobbed locus was discovered. It seems safe to conclude that this species generally carries in its populations, where these have a moderately large effective breeding size, a complex series of alleles at the bobbed locus, similar to those demonstrated.

PLEIOTROPIC EFFECTS OF BOBBED

It is not our intention here to discuss in detail the question of whether so-called pleiotropic effects (see GRÜNEBERG 1943) represent actual multiple or unit gene action. However, in those morphological and physiological effects conditioned by mutant changes there is an actual difference in complexity from locus to locus and among the alleles at one locus. Often the characters conditioned by a single mutant change are not only multiple in the descriptive sense but manifold in terms of their fundamental physiological and even adaptive potentialities. The tracing of the pathways from such characters back to or toward the gene constitutes an important field of research. Until these developmental paths are mapped and probably after it would seem convenient to retain a term for demonstrable multiple effects in contrast to single effects. However, the more careful investigation of many genes now known for one phenotypic effect should demonstrate others, often more important in terms of the physiology of the organism (DOBZHANSKY and HOLZ 1943).

It has been noted that of the two bobbed alleles first described in *D. hydei*, one, Berkeley 28, has slight bristle effect but in addition abnormal abdominal sclerites. In contrast, Wooster 20 shows more extreme bristle change but no abnormal abdominal sclerites. Observations on the many bobbed alleles in the populations studied revealed a complex situation in regard to pleiotropic effects. The characters observed may be listed as follows: reduction in bristle size, abnormal abdominal sclerites, reduction in pigment spots around thoracic bristles, lengthening of larval-pupal period of development resulting in late emergence, lengthening of time to sexual maturity after eclosion, lowered vi-

ability, fecundity, and fertility, and finally lethal effects. Seriation for one of these characters was not absolutely correlated with seriation for others. Thus bristle types of as high grade as 28 might be associated with abnormal abdomen, and types of as low grade as 20 with normal abdomen. Some slight bristle grades showed more larval-pupal retardation than other more extreme types. MOHR (1932) has described a similar lack of correlation in seriation of vestigial alleles for wing reduction and breaks in the second longitudinal vein.

In general it may be stated that the bristle effect was much more often observable than the abnormal abdomen effect and that extreme bristle types were more likely to show abnormal abdomen and other phenotypic characters than slight bristle types. It is not to be understood that all of the alleles placed in one grade in the bristle analysis were indistinguishable on other morphological and physiological grounds. However, due to the complexity of the situation, it was deemed expedient to concentrate on the one character most suitable for metrical analysis. It should also be emphasized that the seriation of a character by counts or measurement may not represent an accurate picture of physiological and developmental grades of equal values. The whole question of thresholds is involved here. Nevertheless, until better tools are at hand it would seem that such measuring of adult characters has value, as long as the method is recognized as giving at least a qualitative and at most only an approximate quantitative picture.

DISCUSSION

We may raise the question as to whether the bobbed situation in *D. hydei* is unique and therefore a detail of passing interest as far as an interpretation of genetic phenomena is concerned. Certainly too many competent observers have worked with many species of Drosophila not to have discovered such a marked phenotypic bobbed complex if it were present. However, the difference in the several species studied is probably not so fundamental as it might appear to be. As mentioned before, 40 mutations to bobbed have been recorded by BRIDGES and others in *D. melanogaster*. These, in general, show a similar phenotypic complex to that found in *D. hydei*. Furthermore, the additive effects of different gene doses of bobbed on the bristle character in *D. melanogaster*, so ably demonstrated by STERN (1929b), have been shown by the author (SPENCER 1930) to hold in *D. hydei* subject to the limitations imposed by chromosome behavior in this species. Using bobbed X chromosomes marked with white, it was shown that XO primary non-disjunction males in *D. hydei* were more extreme bobbed than XX bobbed females. Also XXY primary non-disjunction females carrying bobbed in their X's but the strong non-bobbed allele in the Y were non-bobbed. However, since bobbed alleles in the Y giving phenotypic bobbed combinations have not been discovered in this species, and since it is impossible to build up multiple Y stocks in this species, owing to the absence of secondary non-disjunction, the complete demonstration presented by STERN in *D. melanogaster* is not possible in *D. hydei*.

The absence of phenotypically bobbed males in populations of this species might conceivably be due to the rapid elimination of Y chromosomes carrying

strong bobbed alleles through negative selection. An alternative explanation would be the evolution of some compensatory mechanism in the Y chromosome acting as a suppressor and partially reducing this negative selective factor in a species where the center of the mutational galaxy for bobbed may recently have been brought close to the phenotypic threshold. It might be asked why evolution resulting in adaptive adjustments had partially failed in this case. The answer may well be sought in terms of relative growth rates and compromising adjustments where certain advantages override what may seem to be and in some cases actually are deleterious effects. Furthermore, those changes which might act as suppressors of bobbed in the X may not yet have become established as they apparently have for the Y. On this interpretation the present bobbed condition in *D. hydei* may be thought of as relatively recent in origin.

It seems likely that the genetic structure of a species, through developmental adjustments, may furnish a background against which certain classes of mutations at the same and different loci may be shifted toward or away from the phenotypic threshold or level of expression. It becomes of interest to examine other reduced bristle mutants in this species. By far the commonest class of mutations in *D. melanogaster* are dominant Minute bristle types, lethal in homozygous form and often highly inviable and partially sterile in heterozygous condition. The author has found many of them. But in more extended observations on *D. hydei* no dominant Minutes have been found. It is not unreasonable to suppose that when mutations at these loci occur they are either dominant lethals or are covered up through the evolution of a suppressor mechanism. It may be concluded that the two species, *D. hydei* and *D. melanogaster*, differ in the quantity if not the quality of visible bristle reducing mutations at different loci.

The first case of iso-alleles in Drosophila was described by STERN (1926b). He found that two genes at the ebony locus, designated wild type and e^{12}, were phenotypically identical in homozygous form but gave visibly different phenotypes in heterozygous combinations with another ebony allele. STERN and SCHAEFFER (1943) have recently described iso-alleles at the cubitus interruptus locus in *D. melanogaster*. Here the three wild genes tested showed different potencies when combined with certain cubitus interruptus alleles, particularly if raised at low temperature. Even in homozygous condition, one of the iso-alleles was shown to differ from the others when they were reared at low temperature.

These authors have listed a number of other cases of iso-alleles in Drosophila and in other forms. One of the cases is of particular interest. TIMOFÉEFF-RESSOVSKY (1932) has shown by irradiation experiments that a certain gene at the white locus, extracted from an American strain of *D. melanogaster*, mutates more frequently to or toward white than does a certain gene extracted from a Russian strain. MULLER (1935), by the use of triploids containing two white genes combined respectively with the American and Russian red alleles of TIMOFÉEFF, was able to demonstrate that the American gene had a lower pigment producing potency, was a weaker allele in this respect, than the

Russian gene. He raises the question as to whether the observed difference in mutation frequency of these two genes is real or due to the shift of the whole mutation "spectrum" toward white in the case of the American allele. On this hypothesis more of the mutations of the Russian red gene would fall in the red end of the spectrum, would themselves be iso-alleles. Critical evidence on this point seems to be furnished by the extensive data of TIMOFÉEFF-RESSOVSKY (1933) on mutations of red and of the intermediate alleles at the white locus to white. From his tables we may extract the pertinent data. 48,500 red genes tested mutated 25 times to white; 87,500 genes of intermediate alleles tested mutated 22 times to white. Thus the red mutation frequency in the experiments reported was twice as high as that of the intermediate alleles. Nor can an appreciable error have been introduced by the use of very light alleles where mutations to white might have been overlooked. Only 14,000 of the intermediate genes tested were of alleles lighter than eosin. We must conclude that factors other than or in addition to position in a phenotypic potency spectrum act to determine mutation frequency in different alleles. Otherwise we would have expected higher mutation frequencies to white in the intermediate alleles than in any red, whatever its relation to other reds. However, these studies on mutation frequency and potency have both demonstrated that these two red genes are iso-alleles. It seems likely, as MULLER has pointed out, that both ends of the visible series, red and white, represent in turn a series of iso-alleles grading away from the visible spectrum.

In extensive studies on different Russian populations of *D. melanogaster*, DUBININ and collaborators (1937) found the thoracic color pattern, "trident," in percentages ranging from 2.65 to 39.89. It would seem likely that iso-alleles showing seriation effects in heterozygous combinations might be demonstrated in this complex. The high incidence and many grades of trident in Drosophila stocks have often been observed. In *Drosophila immigrans*, DR. A. H. STURTEVANT first called the author's attention to a series of "net" vein alleles occurring in wild populations. A report on some of these has been presented elsewhere (SPENCER 1940). The variable semi-dominant behavior of some of the stronger of these in certain crosses may be due to the presence of iso-alleles at this locus.

It may be concluded that Drosophila species, differing in their genetic backgrounds, show variations in observable alleles present and in observable mutation rate at certain loci. Actual differences in mutation rate may exist, but this observed difference is apparently due in part to the variable positions in reference to phenotypic thresholds of the means of developmental potencies of mutants at these loci.

Such studies as those reported indicate the complexity of the mutation process and the reservoir of genetic variability lying below the phenotypic surface. The known effects of environmental factors on the expression and suppression of many phenotypic characters and the possibilities of genic interaction in uncovering these hidden mutant galaxies in crosses between strains would seem to provide ample material for preadaptations on which selection may act in the course of evolution.

SUMMARY

Of a large sample of females from an Azusa, Southern California, citrus dump population of *D. hydei*, 3.1 percent were bobbed. Many different phenotypes appeared.

Tests showed: (a) that the variations were due to a complex multiple allelic series at the bobbed locus and not to modifiers; (b) that most of the females tested carried two different bobbed alleles.

Of 106 males from this population 27, or 25.5 percent, produced visibly bobbed daughters when mated to bobbed tester females of grade 20 bristle length. The test indicated that the males carried bobbed alleles capable of seriation when in combination with the tester. But in homozygous condition several of these seriated alleles produced identical maximum bristle types. They are designated as iso-alleles (see STERN and SCHAEFFER 1943).

Bobbed-lethal/bobbed 22 heterozygous tester females were pair-mated to 12 wild Azusa males. Three of the genes tested were recognized as distinct from one another in combinations with bobbed 22; six were so recognized in combinations with bobbed-lethal. One of them produced a lethal compound with the latter.

Using phenotypically different heterozygous combinations with these two testers as a guide, a potency series was constructed with the bobbed-lethal tester at one end and the strongest bristle forming allele seriated at the other end.

Tests of a medium sized population of *D. hydei* from Wooster, Ohio, showed 3.6 percent and 4.8 percent phenotypically bobbed females in two large samples collected.

Of 78 genes chosen at random from this population 36, or 46.2 percent, gave phenotypic bobbed compounds with the same bobbed 20 tester gene used on the Azusa population.

The Wooster sample showed less spread in the allelic series and higher concentrations of a few grades than the Azusa sample. Differences in size and year-round pattern of the two populations probably account for these facts.

Recovery of bobbed alleles from smaller samples of *D. hydei* taken elsewhere is reported. This complex multiple allelic series, with iso-alleles forming lethals at one end and maximum bristle type at the other, is characteristic of this species and not limited to one or a few populations.

Several cases of iso-alleles reported by other workers on Drosophila are compared with the present findings. A discussion of differences in thresholds for phenotypic effects of mutant alleles from species to species and of the possible evolutionary value of iso-allelic series is included.

ACKNOWLEDGMENTS

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ANALYSIS OF MEIOTIC PAIRING IN OLFERSIA AND CONSIDERATION OF THE RECIPROCAL CHIASMATA HYPOTHESIS OF SEX CHROMOSOME CONJUNCTION IN MALE DROSOPHILA[1]

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INTRODUCTION

STEVENS' (1908) initial study of spermatogenesis in male flies disclosed that the mechanism underlying somatic pairing apparently leads directly to parasynapsis of autosomal homologs at the beginning of the spermatocyte growth period. STEVENS emphasized that this course of meiosis in male brachycerous flies is exceptional, for the customary sequence of pre-diakinetic stages appears to be absent and "bivalents" rather than ordinary cross and ring tetrads are formed. Although MORGAN (1912, 1914) shortly thereafter discovered that genetic crossing over is absent in the male of Drosophila, the full significance of STEVENS' discoveries was first appreciated by DARLINGTON (1934a), who clearly demonstrated that the structure of the autosomal bivalents in the male of Drosophila corresponds with a non-crossover tetrad. As STEVENS (1908) and METZ and NONIDEZ (1921) before him suggested, DARLINGTON holds that the autosomes in the male fly conjoin at meiosis by means of exaggerated forces of somatic pairing (a view which is questioned by BAUER [1939] and WOLF [1941]). On the other hand DARLINGTON maintains that unlike the autosomes the sex chromosomes remain paired until meiotic metaphase only because they invariably form reciprocal chiasmata between their homologous inert regions. There is thus envisioned a mechanical dualism in the modes of conjunction of sex chromosomes and autosomes.

The evidence for such reciprocal chiasmata between sex chromosomes in the male of Drosophila, as will be shown in this paper, is both indirect and susceptible to alternative interpretation of less elaborate nature. The purely hypothetical nature of DARLINGTON's interpretation is only too often overlooked, and it is not uncommon for geneticists to express their belief that reciprocal chiasmata between X and Y have been cytologically demonstrated in the male of *Drosophila pseudoobscura* at the least (for example, PHILIP 1935; MATHER 1936; WHITTINGHILL 1937; BROWN 1940; WHITEHOUSE 1942). The fact that in many organisms non-disjunction follows failure of chiasma formation has been generalized into the "chiasma hypothesis of metaphase pairing" (DARLINGTON 1929). But this generalization, which appears to make acceptance of the reciprocal chiasma hypothesis more ready in spite of the obvious and acknowledged absence of chiasmata in autosomal bivalents, is by no means universal in its application. There is now good evidence from mantids (WHITE

[1] This research was supported by a grant from the Penrose Fund of the AMERICAN PHILOSOPHICAL SOCIETY, to which organization grateful acknowledgment is made. A preliminary report will be found in Proc. Nat. Acad. Sci. 30: 50–54, 1944.

1938; HUGHES-SCHRADER 1943a, b), Lepidoptera (*vide* BAUER 1939), a mite (COOPER 1939), a scorpion (PIZA 1939), and a fairly large number of bugs (SCHRADER 1940, 1941) that chromosomes can and do conjoin at meiosis by mechanisms other than chiasmata. To base the means of sex chromosome conjunction in male Drosophila on the chiasma hypothesis of metaphase pairing is no more than to assume a primary mechanism (the chiasma) in Drosophila males which is as much in need of proof as the conclusion (reciprocal chiasmata) itself.

It has been shown that in the fly *Melophagus ovinus* the autosomes of the male physically conjoin by relatively small pairing segments (COOPER 1941). There is no evidence that these conjunctive segments form chiasmata, yet they result in configurations and chromosomal behavior identical with those of the sex chromosome bivalents in male Drosophila for which reciprocal chiasmata have been assumed. Since the comportment of the autosomes of Melophagus indicates that the properties of the sex chromosome bivalent of male Drosophila are not characteristic consequences of chiasma formation alone, the reciprocal chiasmata hypothesis becomes suspect. The following study of a close relative of Melophagus—*Olfersia bisulcata* Macq., a Panamanian fly parasitic on the black vulture—was undertaken with the hope of further elucidating the properties of the conjunctive mechanism apparently common to autosomes in the Melophagus male and the sex chromosomes in male Drosophila. The startling meiotic phenomena discovered in male Olfersia, supplemented by the detailed analysis given below of cytogenetic data on Drosophila, show that there are no longer sufficient grounds for adhering to the reciprocal chiasmata hypothesis proposed by DARLINGTON. Apart from the obvious importance of this conclusion for geneticists working with Drosophila or on problems of crossing over, the present study provides new information on a mechanism of chromosome conjunction at meiosis which does not involve chiasmata. Independently of the reciprocal chiasmata hypothesis, it will be seen that DARLINGTON'S (1929) chiasma hypothesis of metaphase pairing now appears of more limited domain and should be invoked only in those cases where antecedent pachytene and diplotene phenomena justify its application.

MATERIALS AND METHODS

Two males and one female of *Olfersia bisulcata* Macq. were collected from a black vulture (*Catharista urubu* (Viellot)) shot on Orchid Island in Gatun Lake, Canal Zone. One male and the female were fixed for several hours in San Felice, whereas the remaining male was fixed in Allen's B-15 for the same period. The gonads were dissected from the flies in a modified Bělař's salt solution in which the proportions of Na^+ to K^+ in Bělař's original formula are nearly reversed. Peterfi's paraffin-celloidin embedding method was used, and sections were cut from 8 to 15μ in thickness. Stains employed were Heidenhain's iron hematoxylin, Feulgen, and OEHLKERS' (1940) modification of the gentian-violet procedure. Erythrosin was used as a counterstain for some of the gentian-violet preparations, for it brings out the spindle structures clearly. Although fixation with San Felice was satisfactory, the B-15 fixation gave such

brilliant figures with gentian-violet that all but one of the illustrations were prepared from the latter.

Observations were made with Zeiss 3 mm and 2 mm N.A. 1.4 apochromatic objectives, with 20× and 15× Kompens oculars, respectively, and N.A. 1.4 achromatic-apochromatic condenser. Sharpest definition and pleasing contrast of the chromosomes stained with gentian-violet, but not counter-stained, were obtained by employing an Aklo No. 396 heat absorbent glass slip (Corning Glass Works) together with Wratten E22 and Wratten No. 61 filters between the light source and condenser.

THE CHROMOSOME COMPLEMENT

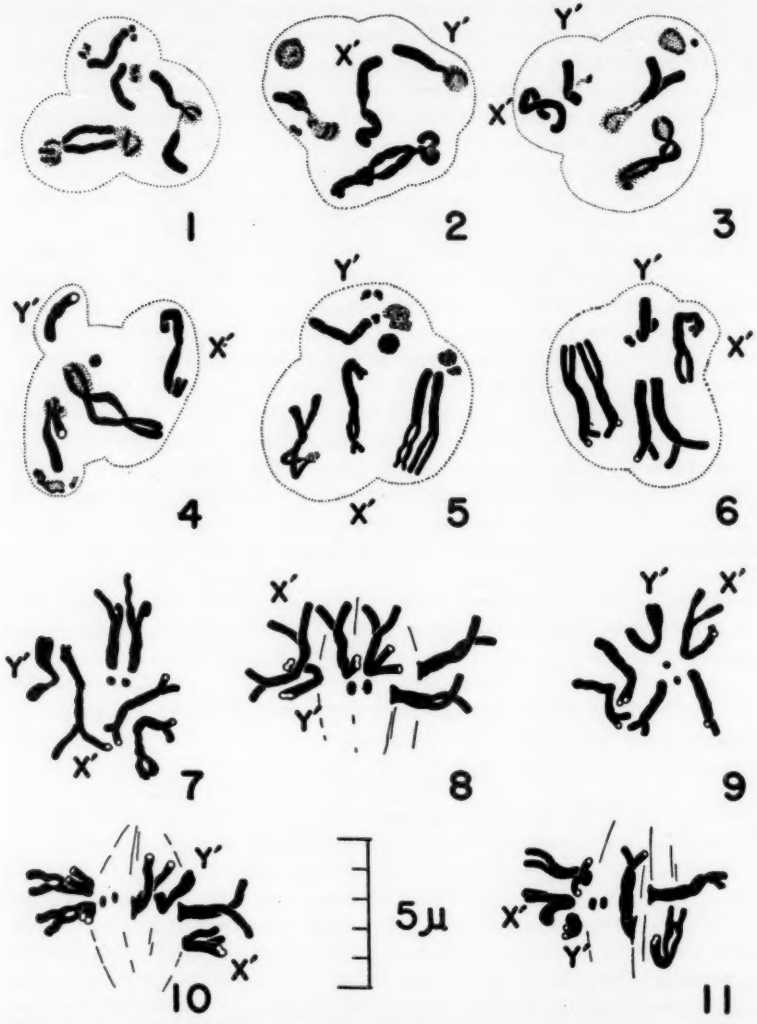
The chromosome garnitures of ten pupiparous flies are now known (COOPER 1941, 1942), and of these Olfersia ($2n=8$) has the smallest number of chromosomes. In the male Olfersia (fig. 7, 9) there are a pair of large V-shaped autosomes with submedian kinetochores, a pair of large and apparently subterminal rods, a pair of small dot-like chromosomes, and a pair of markedly heteromorphic sex chromosomes. The sex chromosomes could not be definitely identified as X or Y because no mitoses were encountered in the ovaries of the female. However, evidence from its pairing behavior at meiosis, as well as heteropycnosis at interphase of meiosis, suggests that the smaller sex chromosome is the Y. Accordingly the large submedian V-shaped sex chromosome will be designated "X'," and the small, nearly median V-shaped chromosome will be denoted "Y'" until such time as the X may be identified with certainty in the female.

It should be pointed out that chromosomes of this fly are especially favorable for the analysis undertaken on two counts. First, the autosomes include the major autosomal types known in Drosophila. Second, the sex chromosome pair morphologically corresponds closely with that of *Drosophila pseudoobscura* race B, the chief difference being that the V-shaped member (Y') is the smaller of the two sex chromosomes in Olfersia.

THE SPERMATOGONIAL PROPHASES

During gonial resting stages the large, slightly staining, spheroidal nucleus contains but few chromatic wisps, a diffuse flocculent mass and a plasmosome. At earliest prophase chromatic segments become visible but show no unequivocal signs of being paired; the flocculency becomes more sparse and the plasmosome dwindles. By mid-prophase (fig. 1) the chromosomes may be individually recognized. The autosomes are somatically paired, whereas the sex chromosomes at most merely occupy the same general region of the nucleus. The sex chromosomes never show close somatic pairing in the gonial prophases while they are condensing or when they have reached the condensed state (fig. 1–6).

The somatic pairing of the large autosomes is quite extraordinary and merits special comment. The rod-shaped autosomes are associated only at their distal regions, the paired regions of the homologs being relatively less condensed than the customarily widely separate medial and proximal portions (fig. 1–4). The



FIGURES 1–11.—Spermatogonial mitosis in *Olfersia bisulcata*. Figures 1–4.—Mid-prophase. Figures 5–6.—Late prophase. Figures 7–10.—Metaphase. Figure 11.—Prometaphase.

V-shaped autosomes likewise show a localization of their somatically paired regions. Their proximal thirds do not pair but form a widely open loop (fig. 1–5). Distal to the central or kinetochore-containing loop the short arms may abruptly twist (from 90° to 180°+) about one another, thereafter somatically pairing more or less along their lengths (fig. 1–4). The long arms likewise twist as they pair for a short region proximal to the central loop. Thereafter the arms once more diverge only to associate again at their distal extremities (fig. 1–4). As in the case of the pair of rod-shaped autosomes, the pairing regions of the V-shaped autosomes appear somewhat less condensed than do the non-paired regions and a flocculent, whey-like coagulum appears to invest the paired regions (fig. 1–4). With the advance of prophase the flocculency vanishes and the paired regions of both the rod-shaped and V-shaped autosomes separate from one another. Correlated with the relaxation of somatic pairing in the rods is the abrupt separation of the chromatids in the distal third to two-fifths of the rod-shaped chromosomes. Ultimate separation and untwisting of the paired regions of the V-shaped autosomes are likewise frequently accompanied by a disjunction of chromatids in both arms but not in the central loop. Somewhat earlier, or at the same time, one or both arms of the X′ chromosome may separate (fig. 4–6). Although a wide separation of chromatids is rarely encountered in Y′, the two chromatids (especially in the long arm) are generally evident in prometaphase and metaphase (fig. 7–11).

The spermatogonial metaphase is like that of most flies, the homologous chromosomes tending to be adjacent to one another on the equatorial plate. Most interesting is the fact that at the equatorial plate stage the sex chromosomes which showed no close association in prophase are as regularly adjacent to each other as are members of the autosomal pairs (fig. 7–11). In anaphase, however, the mitosis is not unlike that of most organisms, for there is no noticeable tendency for homologs to somatically pair. In this respect, as in many others, the spermatogonial mitoses of Olfersia conform to KAUFMANN's (1934) description of mitosis in the ganglion cells of *Drosophila melanogaster* larvae. No differences were observed in the last gonial mitosis, and it would appear that meiotic pairing first occurs in the definitive spermatocyte nucleus following the mitosis. In this respect Olfersia differs from many other flies (STEVENS 1910; METZ 1916, 1926; METZ and NONIDEZ 1921, 1923; etc.).

It is clear that the time of onset and details of somatic pairing are not alike in all Diptera. METZ (1916) generalized the occurrence of somatic pairing at all stages of development and in all tissues examined for many speices, but it is known that somatic pairing is suppressed in the spermatogonia and first spermatocytes of Sciara (METZ, MOSES and HOPPE 1926), is absent in the early oogonia and variable in the early spermatogonia of Tipula, and is not expressed by the supernumerary chromosomes of Tipula (BAUER 1931). SMITH (1942) has recently elaborated a general hypothesis of telophase pairing at the ultimate gonial division. He maintains that a pre-leptotene association in many organisms as well as the somatic pairing of Diptera are consequences of the singleness of chromosomes at anaphase. The assumed singleness of chromo-

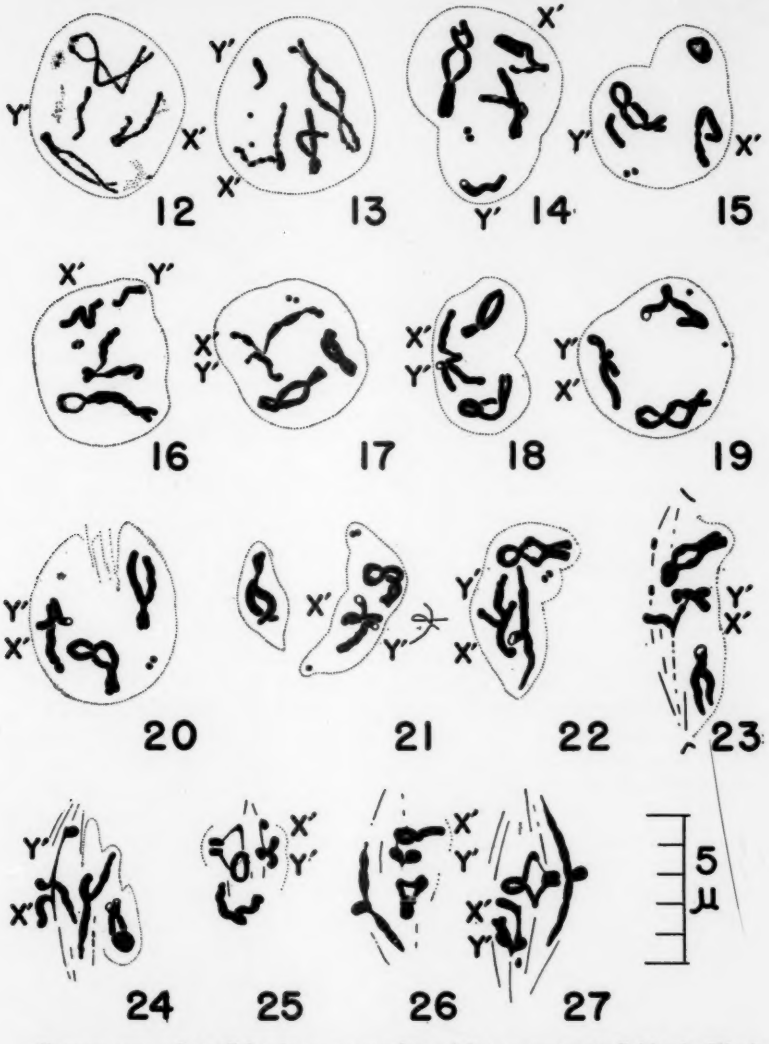
somes and hence pairing are said to occur at the last gonial anaphase in most organisms, but are held a property common to the chromosomes of Diptera "at each and every anaphase." These views are not consistent with the data described above for Olfersia or with published accounts of somatic pairing in many other flies. Without multiplying assumptions, it seems impossible on SMITH's view to account both for the divergence of sister chromatids at late prophase of the gonial divisions and for subsequent failure to pair in anaphase. If the sister chromatids fail to remain associated (and this is a widespread phenomenon in Diptera) at late prophase and metaphase, why do they not reassociate with non-sister homologs? If each chromatid is assumed to be divided and the metaphase chromosome is held to be quadripartite, then somatic pairing should not be found at the succeeding prophase. Without extensive qualification, SMITH's assumptions are likewise out of accord with the facts regarding polytene chromosomes (BAUER 1935; COOPER 1938), multiple association in Culex (BERGER 1937), somatic pairing in polyploid cells (METZ 1916, 1922b, 1925), somatic pairing of chromosomes in ganglion cells of *Drosophila melanogaster* larvae (KAUFMANN 1934),[2] the variable expression of somatic pairing in Tipula (BAUER 1931), the non-pairing of kinetochore regions in Olfersia, Tipula, and Dasyllis (BAUER *op. cit.;* METZ 1922a), and finally the less intense, the frequently variable, or the localized expression of somatic pairing by sex chromosomes (STEVENS 1908; METZ 1914, 1926; COOPER 1941). Whatever the cause of somatic pairing may be, simple non-division of the chromosome does not appear to be the essential factor.

THE MEIOTIC MITOSES

In Olfersia the first spermatocytes apparently occur exclusively in nests of 32 cells each, just as in *Drosophila pseudoobscura* (DOBZHANSKY 1934; STURTEVANT and DOBZHANSKY 1936) and *D. miranda* (DOBZHANSKY 1935), although in the streblid bat-flies, close relatives of Olfersia, six gonial divisions giving 64 cells are the rule (COOPER 1942).

The initial stages of meiotic prophase are not resolvable in the material at hand. Lack of the early synaptic stages will not seriously affect the interpretation of the meiotic mechanism, however, for it is becoming increasingly clear that true leptotene stages may be absent in flies whether or not chiasmata are formed (BAUER 1931; WOLF 1941). Bivalents of clearly defined forms are first encountered in Olfersia at a stage which corresponds with late diplotene of most organisms (fig. 12). In such nuclei all the chromosomes may be recognized, and it is of importance to note that from diplotene through early diakinesis the homologous chromosomes simulate the configurations in the early prophases of spermatogonia described above. Thus the rod-shaped chromosomes are closely associated at their distal thirds or fourths whereas their proximal regions appear to be randomly disposed with respect to each other (fig. 12–19).

[2] SMITH reconciles the evidence of doubleness found by KAUFMANN in homologs which are somatically paired by assuming these cells to be tetraploid. The fact remains that KAUFMANN found no striking evidence for somatic pairing at anaphase but rather that somatic pairing reaches a maximum at prophase.



FIGURES 12–27.—Late diplotene to prometaphase of first spermatocyte division in *Olfersia bisulcata*. Figures 12–13.—Late diplotene. Figures 14–17.—Early diakinesis. Figures 18–19.—Mid-diakinesis. Figures 20–22.—Late diakinesis. Figures 23–27.—Prometaphase. In no case is the sex chromosome pair displaced in the illustration, although when necessary autosomal bivalents are slightly displaced so that they do not overlap.

The homologs of the submedian pair form two loops and a more or less closely paired region distal to the median or kinetic loop (fig. 12–19). The dot-shaped chromosomes generally are separated by a "space" rarely exceeding the diameter of a single dot-like chromosome in width. Expecially striking is the fact the sex chromosomes are not paired (fig. 12–13) and may even lie on opposite sides of the nucleus (fig. 14–15). For the sake of clarity the details of definitive bivalent formation in the sex chromosomes and autosomes will be treated separately.

THE PAIRING OF THE AUTOSOMES

The dot-like pair of chromosomes requires no further comment than given above; its formation of a bivalent parallels that of similar small nearly spherical chromosomes in Diptera. The rod-shaped chromosomes are associated at their distal extremities in much the same manner as the small autosomes at diakinesis, or the proximal ends of the large autosomes, in Melophagus (COOPER 1941). In late diplotene and early diakinesis the paired ends may show a ligher staining gap between the conjoined extremities of the homologs (fig. 12, 14, 16). By mid-diakinesis a line of demarcation is no longer visible between the homologous paired segments (fig. 19–21) but end or side views show the homologs still to be parallel in the paired region as late as metaphase (fig. 22, 23, 29, 31). There is accordingly no evidence for the presence of chiasmata in this bivalent, and on analogy with Melophagus the simplest assumption is that we are dealing with a distal conjunctive segment.

The bivalent formed by the submedian pair of autosomes is most remarkable, superficially appearing to possess at least three chiasmata. Whether or not each of these associations really involves a chiasma is not immediately obvious, but it will be seen that an analysis of these configurations can nevertheless be made. As prophase approaches prometaphase, the bivalent shows three definite loci of association. There is one point of close apposition at roughly the midpoint of each arm. Distal to these two points of apparent contact the short and long arms differ in their pairing. The homologous distal ends of the short arm remain more or less parallel. In the long arm, however, the homologs diverge after their initial contact and form a second loop by pairing once again at their extremities (fig. 12–22). In late diplotene and early diakinesis the loop of the long arm and the paired extremities of the short arm lie at right angles or very obliquely to the kinetic loop (fig. 12–13, 15–18) showing the geometrical relationship so common in bivalents which have an interstitial chiasma in each arm and a terminalized chiasma in one of those arms. But, as is strikingly shown by the data of table 1, the relations of the planes of the loop and paired ends change with the approach of metaphase so that the loop, the parallel arms, or both tend to lie in the same plane as the kinetic loop (fig. 21–23, 25, 29, 31, 33–35). By merging the first two columns (that is, diplotene and diakinesis data) a nine cell table is obtained in which no $m \leqq 5$. There are four degrees of freedom, $\chi^2 = 67.4$, and $P \ll 0.01$. Accordingly we may conclude that the class frequencies of the bivalent configurations for the submedian chromosome pair are not independent of meiotic stage.

TABLE 1

Distribution of V-shaped autosome bivalent-configurations among nuclear stages. Consider the hypothetical axis which passes through the kinetochores and lies in the plane of the kinetochore loop. When the plane of the distal loop in the long arm, or of the paired ends of the short arm, is parallel to this axis it is said to lie in the same plane as the kinetochore loop in spite of obvious lateral flexions of the bivalent concerned. For example, the V-shaped autosome bivalents in figures 25 and 34 are said to have the planes of both arms lying in the plane of the kinetochore loop.

BIVALENT CONFIGURATION	DIPLO-TENE	DIA-KINESIS	PRO-METAPH.	META-PHASE	TOTALS
Class 1: Planes of both distal loops of long arm and distal ends of short arm at right angles or oblique to kinetochore loop (fig. 12, 13, etc.)	24	24	14	4	66
Class 2: Plane of either distal loop of long arm or of distal ends of short arm at right angles or oblique to kinetochore loop, but not both—that is, one of these lies in same plane as kinetochore loop (fig. 22, 27, etc.)	1	1	10	17	29
Class 3: Planes of both distal loops and distal ends of short arm lie in same plane as kinetochore loop (fig. 29, 34, etc.)	0	1	3	18	22
When columns one and two are merged, $\chi^2=67.4$, $n=4$, and $P \ll 0.01$.					117

The simplest interpretation is that both arms undergo a twist at their first locus of association during or before conjunction of their pairing loci. In this respect they correspond with the somatic pairing configurations at prophase in the spermatogonia (see p. 541). With the approach of metaphase, especially as the bivalent orients on the spindle in prometaphase, the divergence of the kinetic loops results in an untwisting moment, so that the planes of the arms come to lie in the same plane as the kinetochore loop. If the average number of twists per bivalent is calculated for each period of prophase, then the relative untwisting of the bivalents with passage from diplotene to late metaphase is given in table 2.

TABLE 2

The relative number of twists per bivalent during meiotic prophase.

MEIOTIC STAGE	NUMBER OF BIVALENTS	AVERAGE NUMBER TWISTS PER BIVALENT
diplotene	25	1.96
diakinesis	26	1.88
prometaphase	27	1.41
metaphase (early)	25	0.76
metaphase (late)	14	0.57

To account for such a reverse rotation of arms distal to chiasmata with divergence of kinetochores would require improbable assumptions regarding the association of strands involved in the hypothetical chiasmata. The interpretation given above is the simplest, and it will be seen that critical evidence from anaphasic disjunction bears out the belief that here we are dealing with pairing segments of chromosomes which do not form chiasmata in the male. Descriptively they may be referred to as "conjunctive segments." Whether or not the twisting is a necessary phase of their function in conjoining the homologs into a bivalent is not known, but it seems unlikely when one considers the similar conjunctive loci of the autosomes of Melophagus (COOPER *op. cit.*).

The Pairing of the Sex Chromosomes

At late diplotene and early diakinesis the sex chromosomes are in most instances undergoing contraction in widely separate regions of the nucleus (fig. 12–15). As contraction progresses, the sex chromosomes come to lie more closely together. At the early stages of their approximation, no pronounced orientation of the sex chromosomes with respect to each other is in evidence (fig. 16). However, in late diakinesis X′ is found adjacent to or actually touching Y′ (fig. 17–19). Such late diakinetic associations as well as those of prometaphase (fig. 20–28) and metaphase (fig. 31–32, 34–35) show that X′ and Y′ conjoin to form a bivalent with regions fairly close to the kinetochore in both chromosomes intimately paired. The pairing segment of X′ appears to be located invariably in the long arm, but Y′ may pair by means of segments in either its short arm (fig. 26, 28, 31, 32) or long arm (fig. 24, 37). X′ long arm by Y′ short arm associations were found in 31 of 41 bivalents which could be analysed with certainty; the remaining ten were X′ long arm by Y′ long arm conjunctions. Quite evidently Y′ possesses a region in both arms homologous with a portion of X′. On analogy with the X and Y chromosomes of *Drosophila melanogaster* (NEUHAUS 1937) and *D. pseudoobscura* (DARLINGTON 1934a), this fact suggests that the Y′ chromosome is the true Y of Olfersia.

Table 3 is a résumé of observations on the progressive pairing of the sex chromosomes at meiotic prophase. $\chi^2 = 57.9$ which, for $n = 4$, corresponds with $P \ll 0.01$. Clearly the relative positions of X′ and Y′ are not independent of nuclear stage. Since no univalent sex chromosomes were encountered at the first meiotic metaphase in an estimated thousand cells, it is justifiable to conclude that the description of sex chromosome pairing given above is correct in all essentials. Namely, the unpaired sex chromosomes at diplotene give rise to sex chromosome bivalents which by metaphase are physically conjoined by short pairing segments located interstitially. As will be seen in the description of anaphase which follows, the conjunctive loci of the sex chromosomes do not differ in their subsequent behavior from those of the autosomes.

Anaphase of the First Meiotic Division

The V-shaped centrioles of Olfersia (fig. 23, 28) are stained only rarely in the preparations at hand. Accordingly the earliest indication of the onset of spindle formation is the assumption of an elongate and often lobulate shape by the late

TABLE 3

Configurations of the sex chromosomes at different meiotic prophase stages.

SEX CHROMOSOME CONFIGURATION	LATE DIPLOTENE AND EARLY DIAKINESIS	MID- AND LATE DIAKINESIS	PROMET.	TOTAL
Sex chromosomes separate and pairing regions not oriented—for example, fig. 12–16.*	16	9	0	25
Sex chromosomes separated by a distance less than length of short arm of Y′ and pairing regions oriented—for example, fig. 17–18, 20–21.	10	11	1	22
Bivalents conjoined—for example, figs. 19, 22–29, 31, etc.	2	15	37	54

$\chi^2 = 57.9$, $n = 4$, $P \ll 0.01$.

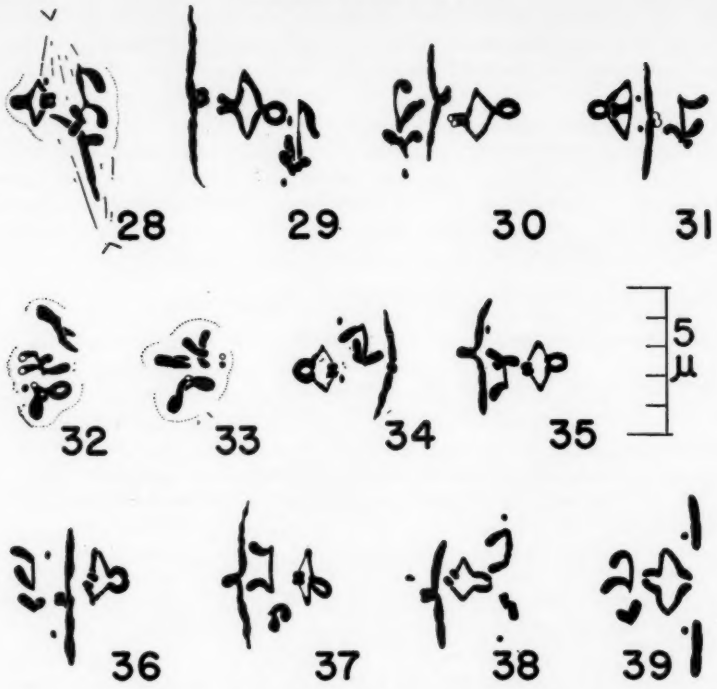
* In cases such as that of figure 16 the two sex chromosomes are separated by less than the length of the short arm of Y′, but the pairing regions are widely separate and not oriented with respect to each other.

diakinetic nucleus (fig. 21–22).[3] The change in nuclear shape during the transition from diakinesis to prometaphase is due to shrinkage of the girth along all but one diameter, not by a process of actual elongation of the nucleus. Nuclear limits vanish wherever they contact the developing spindle, and the bivalents are strewn over the length of the spindle in the initial stages of congression (figs. 23–28).

Although disjunction of the dot-like chromosomes in prometaphase is the rule (fig. 23, 29, 31, 33, 34), a metaphase plate stage for the large chromosomes may be distinguished. At metaphase each bivalent has its cooriented kinetochores approximately equidistant from the equator, this being true also for the disjoined dot-like pair when both its members are visible (fig. 30–36). Apparently metaphase is a stage of short duration, as seems also to be the case in many other Diptera (awakening conflicting reports in descriptions of meiosis in Drosophila males).

The time of onset of early anaphasic disjunction is not synchronous among the bivalents, but seems to be inversely correlated with the absolute physical length of association in each bivalent. Thus the dot-like pair separates in prometaphase and the sex chromosome bivalent generally disjoins while the rod-chromosome bivalent and V chromosome bivalent are still in metaphase (fig. 30, 37). Although the rod bivalent remains closely conjoined during the initial opening of the paired arms of the V chromosome bivalent (fig. 36, 38), it nevertheless undergoes disjunction and poleward movement while the V bivalent is still in its early phases of disjunction (fig. 38–40). Late anaphases show that the chromosomes tend to reach the poles as a group in spite of the

[3] Division of the nucleus into two separated lobules as in figure 21 was noted only infrequently.



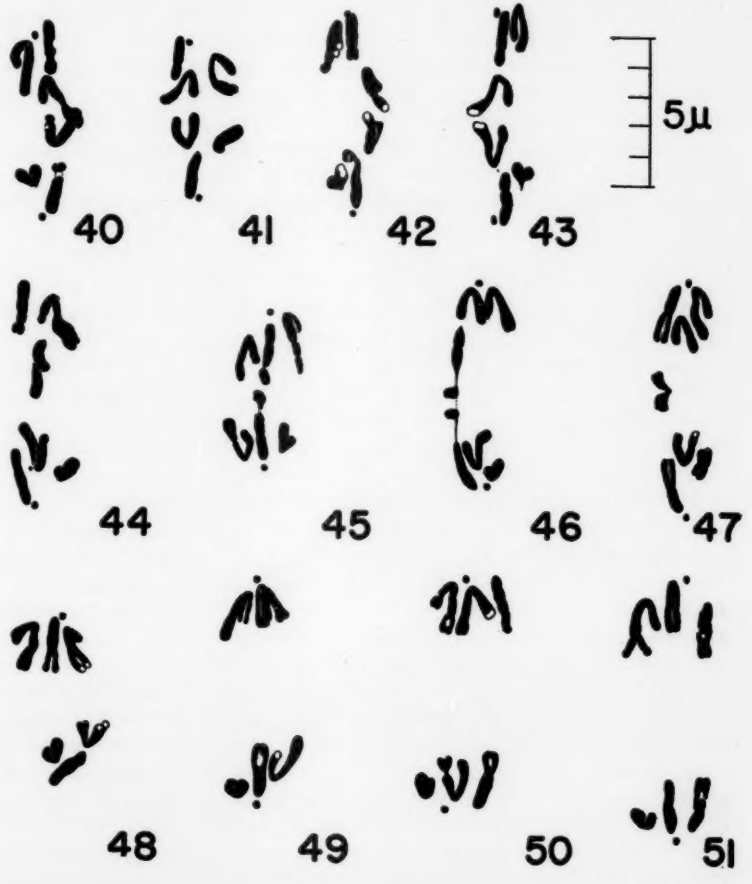
FIGURES 28–39.—Prometaphase to early anaphase of first spermatocyte division in *Oifersia bisulcata*. In all figures, excepting 32, 33 and 35, X′ is directed toward the upper pole. Figures 28–29.—Prometaphase. Figures 30–37.—Metaphase. Figures 32–33.—Metaphase in polar view. Figures 38–39.—Early anaphase. Where necessary bivalents have been laterally displaced to avoid overlaps.

definite order of completion of disjunction (fig. 40–51). At early telophase the distal ends of the arms of the X′ and V chromosomes, and the distal ends of the rod chromosomes show their chromatids to be disjoined (fig. 52, 53). The arms of Y′ occasionally evince a split delimiting the component chromatids of one (fig. 49) or both arms (fig. 47), but the chromatids do not separate widely. No evidence of duality was observed in the dot-like univalents, perhaps owing merely to lack of resolution.

Disjunction of the Large Autosomal Bivalents

Considering the evidence from prophase that the rod, V, and sex chromosome bivalents are not conjoined by chiasmata, the actual disjunction figures of these bivalents merit special attention. For this reason it is regrettable that the precise details of initial disjunction could not be satisfactorily deciphered for the conjoined segments of the rod chromosome bivalent. Whether, as is not infrequently the case for the short arm of the V chromosome bivalent, there is

a regular or occasional twisting of homologs at the proximal ends of the conjoined segments at metaphase could not be decided because of the intimacy of the association. That such may be the case is hinted by some figures (namely, fig. 22, 23, 31, 36), as well as by the fact that the conjunctive segments appear to disjoin first at their distal extremities (fig. 38). In any event final separation is achieved with more or less parallel disjunction of the conjoined segments, between the proximal ends of which there may persist a faint thread of dubious significance (fig. 46). The separating ends of the rod chromosome bivalent show no separation of sister chromatids at early anaphase (fig. 45), but one



FIGURES 40–51.—Anaphase of the first spermatocyte division in *Olfersia bisulcata*. X′ is at the upper pole in all figures. Where necessary, bivalents have been laterally displaced to avoid overlaps.

(about 20 percent) or both (about 13 percent) of the univalents from this bivalent may have split distal ends at late anaphase (fig. 40, 42, 44, 48–51); by telophase all are split (fig. 52, 53). In one instance the conjunctive segments failed to disjoin at what appears to be the distal end. In this isolated case a fracture and separation of both univalents from their conjunctive segments seems to have resulted (fig. 47).[4]

At metaphase and early anaphase the V chromosome bivalent tends to complete rotation of whatever twists are residual in the conjoined segments (fig. 29–35). Disjunction of the short arms results in parallel to oblique displacement of these arms without any evidence of terminalization or exchange of partners (fig. 36, 38, 39). The loop in the long arm similarly undergoes disjunction without terminalization of the proximal association. Characteristically, the proximal association in the long arm disjoins first (fig. 36, 38), followed by separation of the terminal association (fig. 38, 39). In some instances the rotation of one of the conjoined arms is not complete at the time of disjunction, resulting in configurations such as that of figure 40. Customarily there is no visible separation of the chromatids in the univalents of the V chromosome bivalents in early and mid-anaphase (fig. 40–47), but in late anaphase and early telophase the chromatids separate in one or both arms (fig. 48, 51, 52, 53).

Disjunction of the Sex Chromosome Bivalent

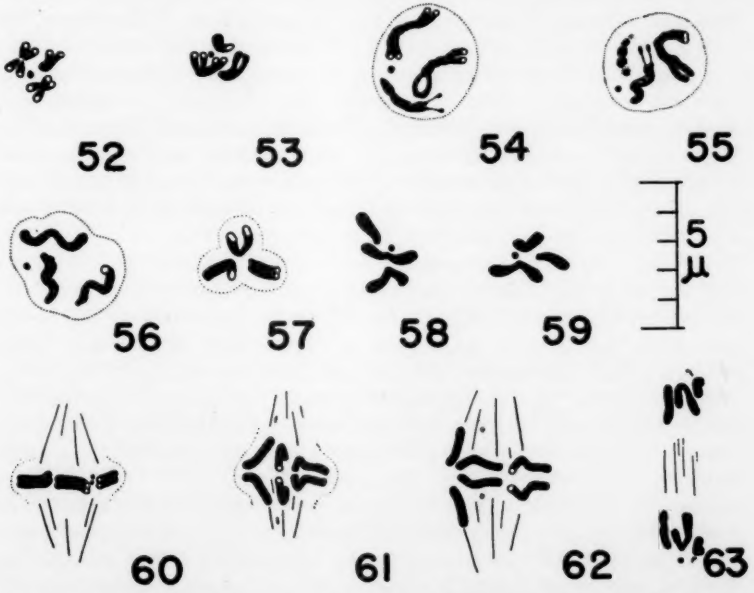
The fact that X′ and Y′ pair in prophase at a time when they are already markedly condensed may be taken as conclusive evidence that they are not conjoined by chiasmata. Nevertheless, the disjunction of the sex bivalent follows in detail the course of events described by DOBZHANSKY (1934), DARLINGTON (1934a), and KOLLER and TOWNSON (1933) for the sex chromosome bivalent in *Drosophila pseudoobscura*, and by DOBZHANSKY (1935) and KOLLER (1939) for the X¹Y bivalent of *D. miranda*, where reciprocal chiasmata are supposed to occur. During congression and metaphase the kinetochores of the X′Y′ bivalents appear to be under tension, as evidenced by the attenuation of the points of apparent spindle fiber insertion (fig. 23, 24, 26–29, 31, 34, 35). The limb of X′ between the kinetochore and the locus of conjunction with Y′ becomes drawn out (fig. 23–25, 27–29, 31, 34–35), although the portion of this arm distal to the locus of conjunction remains unaffected (figures cited). Only rarely does the proportionately stouter segment between the kinetochore and conjunctive locus of Y′ show pronounced attenuation (fig. 24). At early anaphase there appears to be a parting of the conjoined segments without any appreciable alteration in the relative dispositions of the limbs distal to the loci of former conjunction (fig. 30, 36, 37, 39). As anaphase progresses, the attenuated arm of X′ shortens, and the general appearance of the poleward moving sex chromosomes becomes normal in all respects (fig. 38, 40–46). By late anaphase one or both arms of X′ show a separation of chromatids to have occurred (fig. 44, 48–51). In telophase the chromatids of both arms distinctly

[4] True non-disjunction has been observed in Melophagus where similar conjunctive segments are involved (unpublished data).

separate (fig. 52, 53), and it is in this condition that X′ enters interphase. As noted above, Y′ rarely shows more than a line of separation between its component chromatids at late anaphase (fig. 47, 49).

Interphase and the Second Meiotic Division

At early interphase, separated chromatids show characteristic patterns in the rod and V chromosome and X′ univalents. Thus the distal extremities of the rod univalents often appear negatively heteropycnotic, the chromatids in this region being divergent or parallel, and the ends bead-like (fig. 54, 55). The V chromosome univalents show both arms divided, and frequently the chromatids of the long arm form a loop through contact of their ends (fig. 54). This condition is not infrequently noted during late anaphase, although it is only poorly represented in one of the figures illustrated (fig. 51). As noted above, X′ has both arms prominently split at early interphase (fig. 54). The structure of Y′ is difficult to decipher, but it may be stated generally to show differential heteropycnosis (fig. 55). The dot-like autosome remains plainly visible throughout interphase, but shows no eccentricity of behavior. The chromosomes do not fade from view during interphase. Onset of the second meiotic division is



FIGURES 52–63.—Telophase I, interphase, and second spermatocyte division in *Olfersia bisulcata*. Figures 52–53.—Equatorial views of polar telophase I groups. Figures 54–55.—Interphase nuclei. Figures 56–57.—Second prophase. Figures 58–60.—Second metaphase. Figures 61–63.—Second anaphase. Univalents have been laterally displaced to prevent overlaps only in figures 60–62.

forecast by the shortening of the chromosomes and the parallel reassociation of their chromatids where formerly separate (fig. 56, 57). The univalents form a flat metaphase plate (fig. 58–60) and initiate anaphase more or less simultaneously. The chromatids of the dot-like chromosome, as would be expected, complete their separation first. The other chromosomes appear to achieve their early anaphase separation by both pronounced parallel displacement of sister chromatids and an increasingly more rapid separation of kinetochore regions (fig. 60–63).

Chiasmata and Conjunction of the Large Autosomes

The data of anaphasic disjunction are in agreement with the conclusion that in Olfersia conjunction of the rod chromosomes in meiosis is independent of chiasmata. Suppose, for example, that the associations in this bivalent were due to a single, sub-terminal chiasma. The disjunction at anaphase should then result in terminalization with consequent separation of chromatids distal to the point of exchange. Actually, however, disjunction does not result in terminalization, for the conjoined chromosome segments part distally prior to or simultaneously with disjunction of the most proximal point of association. Furthermore, separation of sister chromatids distal to the supposed point of exchange, resulting in bifurcation of the distal ends of the chromosomes, does not occur in the univalents at the time of disjunction. The chromosomes are not so small as to make observation of such a separation of chromatids impossible if it occurred, for later in anaphase such a separation of chromatids is characteristically found. However, separation of chromatids at this time does not show this to be the result of disjunction of conjoined segments, for at late anaphase (fig. 49) and early telophase even the short arm of X′ may have its chromatids separated. This is an important and relevant point, for the short arm of X′ has never been observed to conjoin with Y′.

Furthermore, a cross configuration has never been observed at the region of conjunction in this bivalent. This fact requires an emendation of the simple assumption that a single, subterminal chiasma is involved. Namely, it would have to be asserted that the portions of the homologs distal to the assumed chiasma remain paired over a sizeable length of chromosome at the very time (late diplotene through metaphase) the proximal three-fourths of the homologs evince no mutual attraction. Such an admission would in itself remove the theoretical requirement for the postulated chiasma. If the distal fourths of the homologs can remain conjoined through metaphase without chiasmata, then segregation is ensured. The disjunction figures actually found at anaphase are those to be expected in such an event. Accordingly, if the chiasmatype hypothesis is not to be modified by improbable subsidiary qualifications, then the bivalent must be interpreted as either conjoined by multiple chiasmata or by some mechanism other than chiasmata.

Assumption of an even number of randomly disposed chiasmata within the segment, or of an odd number of such multiple chiasmata, awakens the same sort of difficulty as the apparently invalid postulate of a single chiasma. The remaining chiasmatype interpretation is that conjunction in this bivalent is

brought about solely by reciprocal chiasmata as envisioned by DARLINGTON (1934a, 1934b, 1935, 1937, 1939b) for the sex chromosomes of male Drosophila. However, this assumption is not as simple as it appears. (Its validity as a hypothesis accounting for the conjunction of sex chromosomes in Drosophila will be examined in another section of this paper.) Unless the two-strand doubles involved in this hypothesis are further specified to have twists (that is, "chiasma direction") of the crossover chromatids reversed in direction and passing through approximately 180° at the two levels of exchange and unless sister strand relational coiling is absent between the loci of exchange, then chromatid locks and chromosome locks (terminology of SAX 1936) should occur. The reciprocal chiasmata would have to be entirely of the above restricted class because neither type of lock has been found, although both kinds would give easily recognizable configurations at anaphase. But the assumption that all the rod chromosome bivalents are of the requisite class implies the simultaneous existence of absolute and negative chromatid interference, localization of the chiasmata, and a restriction of relational coiling. That no line of separation corresponding with the rift between sister chromatids is laterally visible between the conjoined segments at metaphase affords but scant direct evidence that we are not dealing with the specified reciprocal exchange. But the fact that the paired segments do not form an open loop visible in polar view completes the vitiation of this already hopelessly complicated hypothesis, for further assumptions would have to be manufactured to account for the lack of such a loop.

Moreover, the pairing mechanism of the sex bivalent of Olfersia described above (page 546) and the pairing of the autosomes of Melophagus (COOPER 1941) make it obvious that conjunction of restricted chromosome segments may occur without benefit of chiasmata. The assumption of reciprocal chiasmata in the rod chromosome bivalent of Olfersia therefore falls of its own weight, and we may conclude that here, as in the cases mentioned above, we are dealing with what may be descriptively termed a "conjunctive segment" free of chiasmata. Comparison with the V chromosome bivalent adds further support to this conclusion.

Expecially striking in the case of the V chromosome bivalent is the uncomplicated separation of the proximal association in the loop of the long arm. The points of conjunction merely part from one another, there being no visible exchange of chromatids as would have to occur were a single chiasma the mode of conjunction at this locus. Accordingly we are reduced once again to a choice between postulating a reciprocal exchange of specified architecture, or granting the occurrence of conjunction without chiasmata. Furthermore five chiasmata, two pairs of reciprocal exchanges and a terminalized single exchange are required to account for the anaphasic disjunction configurations of the short arm association and long arm loop purely on the chiasmatype hypothesis. Since the arguments given above for the rod chromosome bivalent are all applicable to this case as well, the simplest conclusion is that meiotic conjunction is similar in mechanism in both of the large autosomes and in the sex chromosomes. It is therefore concluded that in the male Olfersia segregation is guar-

anteed for autosomes and sex chromosomes alike by a conjunctive mechanism which does not involve chiasma formation.

THE RECIPROCAL CHIASMATA HYPOTHESIS OF SEX CHROMOSOME CONJUNCTION

The metaphase pairing of the sex chromosomes in the Drosophila male presents a problem which is not only interesting in itself, but also bears directly on the meiotic mechanism in general. DARLINGTON (1931a, to date) has put forth the hypothesis that the X and Y chromosomes in Drosophila males are conjoined at meiosis through invariate, reciprocal chiasmata which are for the most part genetically undemonstrable. The argument of this hypothesis, to be treated in detail below, rests chiefly on the observation that the X and Y chromosomes form an intimate interstitial union for a short distance of their lengths. This fact loses much of its significance for DARLINGTON'S hypothesis in view of the findings reported above for Olfersia, as well as those for Melophagus (COOPER 1941). These new data are entirely consistent with the view that meiotic conjunction of homologous chromosomes in male Diptera may be brought about by intimate association of small regions devoid of chiasmata. Accordingly it is necessary to reexamine the premises and evidence on which the reciprocal chiasmata hypothesis is based.

The chiasmatype hypothesis, first propounded by JANSSENS (1909) and further elaborated by WILSON and MORGAN (1920), held that the chiasmata are the direct consequences of genetic crossing over. It is DARLINGTON'S noteworthy distinction that he more than any other, through a series of brilliant observations and inductions (1930, 1931b) brought this hypothesis to its present status as one of the underlying theories of cytogenetics. Coincidentally with his development of the chiasmatype theory, DARLINGTON propounded (1929) a new hypothesis of metaphase pairing at meiosis. Since at diakinesis and first metaphase, in the forms he studied, chromatids are held together only in pairs, and chromosomes are held together only by means of exchanges of partners (that is, chiasmata) among these pairs of chromatids, DARLINGTON drew the conclusion that only chiasmata provide a mechanism for conjunction of homologs after pachytene. The well known fact that males of the genus Drosophila do not show genetic crossing over would seem to negate the generality of this subsidiary hypothesis. DARLINGTON (1931a), however, argued that since all forms satisfactory for chromosome study have their bivalents conjoined at first metaphase by chiasmata, the Drosophila male alike must have conjunction through chiasmata. Whereas the chiasmata (as inferred from crossing over data) in the female of Drosophila are distributed over the length of the chromosomes, those of the male, where interstitial, must be reciprocal and localized in genetically neutral segments of the chromosomes—namely, in the vicinity of the kinetochores. Thus autosomes and sex chromosomes alike were held by DARLINGTON to be conjoined by reciprocal chiasmata of such disposition that crossing over would be, for the most part, genetically undetectable. Cytological evidence in support of this interpretation was notably lacking at the time, but to certain of STEVEN'S (1908) figures of Drosoph-

ila DARLINGTON could give an interpretation compatible with his *ad hoc* hypothesis. It is true that METZ' (1926) figures of the autosomes of *Drosophila willistoni* and *D. pseudoobscura* (=*obscura* of METZ) were in obvious disagreement with DARLINGTON's hypothesis, but these observations DARLINGTON held less decisive than those of STEVENS.

Nevertheless METZ proved to be correct, a fact which both DARLINGTON (1934a) and DOBZHANSKY (1934) later affirmed. The autosomes of *Drosophila pseudoobscura* form bivalents which at metaphase have their kinetochores directed to opposite poles, but along their distal lengths the four chromatids are paired parallel to one another. A clear space customarily shows between the parallel limbs of the two chromosomes of each autosomal bivalent. On the other hand, the sex chromosomes possess a joint interstitial connection for a short segment of their lengths. The contrast between the two types or bivalents led DARLINGTON (1934a) to conclude that the autosomes are devoid of chiasmata and conjoined through specially exaggerated forces of somatic pairing (compare STEVENS 1908; METZ 1916; METZ and NONIDEZ 1921) whereas the sex chromosomes conjoin by reciprocal chiasmata as he had earlier postulated. The minimum specific assumptions of the reciprocal chiasmata hypothesis for conjunction of the sex chromosomes in male Drosophila appear to be the following:[5] (1) the chiasma hypothesis of metaphase pairing is valid for the sex chromosomes of the Drosophila male, despite the fact that it is not so for the autosomes; (2) chiasmata conjoining the inert homologous regions of the sex chromosomes are regularly present at meiosis in the male (see 3b *et seq.* below); (3) genotypic control guarantees in the male: (a) the complete suppression of all meiotic crossing over between autosomes, (b) the almost invariable production of two and only two chiasmata per sex bivalent, (c) an absolute negative chromatid interference in the homologous inert regions of the sex chromosomes so that if two chiasmata are formed, they are without exception reciprocal, (d) a reversal of chiasma direction at the second chiasma (see p. 553), (e) the suppression of sister chromatid coiling between chiasmata (see p. 553).

It is clear that such an elaborate hypothesis concerning the mechanism of sex chromosome conjunction is required only if assumptions (1) and (2) are valid. What appears to be the principal genetic and cytological evidence relevant to these two assumptions will now be examined.

Consideration of the validity of the chiasma hypothesis of metaphase pairing for Drosophila.—GOWEN (1928, 1933) has shown that there is a high rate of non-disjunction correlated with the almost complete suppression of crossing over in female *Drosophila melanogaster* homozygous for *c3G*. At first thought this might appear as strong support for DARLINGTON's (1929) chiasma hypothesis of metaphase pairing, but in fact there are two conditional points which must be stressed and which allow of alternative interpretation. First, the time of action of *c3G* appears to be at meiosis (GOWEN), but not necessarily at the time of crossing over (chiasma formation). For example, *c3G* may act by sup-

[5] Relevant discussions or explicit treatment of most of these assumptions will be found in DARLINGTON 1931a, 1934a, 1934b, 1935, 1937, 1939b.

pressing a necessary antecedent condition to crossing over. If *c3G* shortens the time available for leptotene pairing so that meiotic synapsis is rarely normal in the female, then the consequent non-disjunction would at most indicate only a need for synapsis in the female rather than an absolute requirement of chiasmata for bivalent conjunction and subsequent regular disjunction. Second, while crossing over (hence chiasma formation) is virtually completely suppressed and there is attendant non-disjunction, maturation nevertheless favors the production of normal gametes. There is a considerably greater number of normal haploid eggs (about 18 per cent more) formed than can be accounted for by random distribution of the chromosomes alone. Hence, since homozygous *c3G* does not cause non-disjunction in the male of either sex chromosomes or autosomes (GOWEN 1928, 1933; DARLINGTON 1934a), it seems reasonable to suppose there is some meiotic factor common to male and female meioses which tends to guarantee some measure of pairing and normal segregation in spite of suppression of crossing over. That disjunction of the sex chromosomes in the male is totally unaffected by homozygous *c3G* strongly suggests that X and Y do not require chiasmata for conjunction and hence segregation. DARLINGTON (1931a, 1934a, 1937), however, prefers to suppose that *c3G* suppresses both pairing and crossing over, but that in the male genotypic control (assumption 3 above) inhibits the anticipated effects of *c3G* on crossing over in the sex chromosomes as well as on the simple, non-chiasma pairing of the autosomes of the male.

Be that as it may, it is now certain that crossing over between sex chromosomes in the female is not always required for their normal segregation. STURTEVANT and BEADLE (1936) found that in In(1)dl-49/+ females about half of the sex chromosome tetrads underwent no exchange. Despite this gross failure of detectable crossing over, no matroclinous female exceptions appeared among 3,238 daughters. Although BROWN's (1940) observations show crossing over to be very infrequent in the inert region, it might nevertheless be contended that undetectable exchanges in the inert regions had been responsible for the resulting normal disjunction. However, STURTEVANT and BEADLE showed this to be implausible, for heterozygous inversions upsetting homologies within the inert regions themselves (In(1)Df(*bb*), In(1)*sc-8*, In(1)Df(*sc-8*)) result in considerable numbers of non-crossover tetrads and yet fail to increase the matroclinous female exceptions above the normal rate. Crossing over is therefore not essential for normal disjunction of the sex chromosomes in the female of *Drosophila melanogaster*.[6] Since crossing over (hence chiasmata) is not a prerequisite to regular disjunction of the sex chromosomes in female Drosophila, or of the autosomes in Drosophila males, it is not likely that crossing over (hence chiasmata) is necessary for metaphase pairing of X and Y in the male.

Accordingly assumption (1) that the chiasmata hypothesis of metaphase pairing is valid for the sex chromosomes in male Drosophila may be concluded to be unsupported and not required by genetic data.

[6] Experiments by the author, shortly to be published, demonstrate that exchanges in the small right arm of the X cannot be held accountable for normal segregation in these experiments.

Consideration of genetic evidence relative to the regular occurrence of crossing over at meiosis between the sex chromosomes of the male Drosophila.—There is no longer any doubt that X and Y can crossover in the male, for this has been shown by the work of STERN (1929, 1936), PHILIP (1934, 1935), STERN and DOAN (1936) and NEUHAUS (1937). Excepting PHILIP's data for the moment, and STERN's (1936) instances of crossing over in somatic cells, the remaining cases show detectable exchanges between X and Y to occur with a frequency of roughly $2\text{–}8\times10^{-4}$ in the male. Furthermore, crossovers tend to occur in small clusters in the progeny of individual males suggesting, as STERN and DOAN point out, that the exchanges occur not at meiosis but probably during gonial divisions.[7] Discovery has been made of similar spontaneous but exceedingly rare crossing over between autosomes in males of *Drosophila melanogaster* (MULLER 1916; BRIDGES and MORGAN 1919; PATTERSON and SUCHE 1934), *D. simulans* (STURTEVANT 1929), *D. virilis* (KIKKAWA 1933) and *D. ananassae* (MORIWAKI 1937). MULLER's case requires the original crossover to have occurred in an embryonic cell[8]. As BRIDGES and MORGAN point out, if the 43 apparent crossovers between purple and vestigial in a total of 573 offspring which they obtained from a single male are truly crossovers, then, as with MULLER's case, their occurrence was probably due to an exchange which occurred in a cell far antecedent to normal meiosis. Likewise crossovers between homologous autosomes which have been induced, by heat or X-rays, in Drosophila males tend to occur rarely, in clusters from individual males, and with unequal reciprocal classes (review in WHITTINGHILL 1937). Thus autosomal as well as X-Y crossing over occurs in the male, but both types of crossing over probably occur not at meiosis but in gonial or gonial-precursor cells—that is, they are due to mitotic crossing over (WHITTINGHILL 1938). Since the autosomes of those Drosophila males which have been studied do not show any evidence of chiasmata at meiosis,[9] the occurrence of rare crossing over between the sex chromosomes in males cannot be taken as implying the occurrence of chiasmata in the sex chromosomes at meiosis of spermatogenesis. Expressed otherwise, the capacity for regular crossing over at spermatogenesis is not prerequisite to gonial or somatic crossing over in the male. Nevertheless the structural changes resulting from the X-Y crossovers mentioned above have been presented by DARLINGTON (1937) as support for his assumptions. It is clear from what has just been said that they furnish no such support.

[7] WHITTINGHILL's (1937) suggestion that spermatid multiplication could also account for clustering of rare crossovers is improbable, as he himself appears to feel. Such an explanation is not applicable to the female for which only embryonic or oogonial crossing over may be invoked to account for similar data.

[8] STURTEVANT (personal communication) believes MULLER's case is more likely a consequence of mutation than of crossing over.

[9] See figures of *D. funebris* (METZ 1926); *D. melanogaster* (STEVENS 1908; METZ 1926; GUYÉNOT and NAVILLE 1929; ZUITIN 1929; HUETTNER 1930; WOSKRESSENSKY and SCHEREMETJEVA 1930); *D. miranda* (DOBZHANSKY 1935, 1937; KOLLER 1939); *D. pseudoobscura* (METZ 1926; KOLLER and TOWNSON 1933; DARLINGTON 1934a; DOBZHANSKY 1934; STURTEVANT and DOBZHANSKY 1936); *D. virilis* (METZ 1926); *D. willistoni* (METZ 1926).

PHILIP (1934, 1935) maintains that her cytogenetic experiments demonstrate reciprocal exchanges to occur between X and Y chromosomes in male *Drosophila melanogaster* with a frequency of approximately $3\text{–}7\times10^{-4}$ (that is, of the same order of magnitude as apparent single exchanges in the male). Since her work was done before NEUHAUS' (1936b, 1939) findings to the contrary, PHILIP assumed the normal allele of bobbed to be located in the long arm of the Y chromosome (Y^L), that crossing over between Y^L and X may take place to the left of bobbed, and lastly that Y crosses over with X for the most part, if not exclusively, through exchanges in Y^L. The latter assumption was also made by DARLINGTON (1931a) who, to account for the formation of STERN's (1929) $\widehat{XY}'$ ($=\widehat{XY^L}$) chromosome in the male, held that it arose through an "inverted chiasma" between X and Y^L. KAUFMANN (1933) quite properly pointed out that such an aberrant exchange would nevertheless fail to give $\widehat{XY}'$. DARLINGTON's escape from the dilemma was not to accept the obvious alternative suggested by KAUFMANN—that is, that an ordinary exchange between Y^s and X may give rise directly to $\widehat{XY}'$—but to shelter his initial assumption under an additional one. He now postulated (1934b) crossing over to occur within and between the "attachment chromomeres" (=kinetochores) of X and Y which were further assumed to have paired in an inverted way! PHILIP (1935) avoided such a complicated explanation by assuming that the small arm of X to the right of the kinetochore (KAUFMAN, 1934; PROKOFIEWA 1935) is homologous with part of Y^L and inverted with respect to Y^L. If this were so, a simple crossover between X^R and Y^L would give $\widehat{XY}'$ (PHILIP terms this an "inverted crossover"). While PHILIP's suggestion merits consideration, NEUHAUS (1937) has given good reason to believe that in the male effectively single exchanges are far more frequent between Y^s and X than Y^L and X.

With regard to PHILIP's demonstration of double crossing over between X and Y, some hesitance must be felt in accepting these data for it is not clear from her unfortunately ambiguous statements that the cytological and genetic tests were sufficiently rigorous to justify the conclusions she has drawn. But if it is assumed that she has indeed discovered double reciprocal exchanges between X and Y in the male, it is by no means shown that these followed from meiotic crossing over. There is no mention of whether or not the rare exceptions tended to occur in small clusters, but as BAUER (1937) points out, the numerical inequalities between her experiments suggest that PHILIP's crossovers are the results of gonial exchanges. That double "crossing over" may occur mitotically has already been made probable by BRIDGES and MORGAN's (1919) "doubles" between vermilion and sable which appeared in every offspring of a single female, as well as STERN's (1936) analysis of somatic crossing over. Although DARLINGTON (1935, 1937, 1939b) and PHILIP both consider her data as strong support for the reciprocal chiasmata hypothesis, such detection of rare crossovers between X and Y in the male can not be brought forward as proof of chiasmata at meiosis, as BAUER (1939) has already pointed out.

One additional genetic problem concerns the regular disjunction of Y from XX in females. It is DARLINGTON's (1937) assumption that the genotypic determination of reciprocal chiasmata between X and Y does not occur in the female. In this event, if crossing over is necessary for regular disjunction, as DARLINGTON maintains, the rate of detachments of $\widehat{XX}$ should be very high with resultant formation of $\widehat{XY}^L$ and $\widehat{XY}^s$ in large and approximately equal numbers. Such detachments of $\widehat{XX}$ owing to crossing over of $\widehat{XX}$ with Y are rare and occur about as frequently as does gonial crossing over between X Y in the male, having a frequency of approximately 6.6×10^{-4} (KAUFMANN 1933; NEUHAUS 1936b; etc.) Thus DARLINGTON is faced with either granting that regular disjunction may occur without benefit of chiasmata, as has been shown probable on other grounds, or supposing that the efficient genotypic mechanism resides within the Y chromosome itself and does cause reciprocal chiasmata formation between Y and X in the female. Briefly, the Y must be conceived as almost invariably eliciting reciprocal exchanges with the X at meiosis whether in male or female. This seems very unlikely.

It may be concluded, therefore, that no genetic data are available which require assumption (2) that chiasmata are regularly formed between the homologous inert regions of X and Y in the male. Indeed there is no satisfactory genetic evidence that chiasmata ever occur between the sex chromosomes of the male at meiosis.

Consideration of the validity of the cytological evidence for reciprocal chiasmata in the Drosophila male.—Although, as has been shown above, the genetic data neither require nor support the primary assumptions of the reciprocal chiasmata hypothesis, it remains possible that cytological phenomena make this hypothesis necessary. Consideration of the cytological literature does not show this to be the case.

Shortly after the publication of DARLINGTON's (1931a) initial hypothesis of reciprocal chiasmata in both autosomes and sex chromosomes of the male Drosophila, KOLLER and TOWNSON (1933) recorded the occurrence of possible chiasmata in both the autosomes and sex chromosomes of *Drosophila pseudoobscura* males. The later work of DARLINGTON (1934a) and DOBZHANSKY (1934) showed the autosomes to be devoid of chiasmata, and the evidence for chiasmata in the sex chromosomes to be purely indirect. KOLLER's (1939) recent account of reciprocal chiasmata between X^1 and Y in *Drosophila miranda* males must also be discounted, for the author states that the $X^1 X^2$ Y chromosomes "are too small to allow a critical study." Indeed, KOLLER could not even decide whether a sex chromosome trivalent was formed in the interracial hybrids he studied. Accordingly the observations on the sex chromosome bivalent of male *Drosophila pseudoobscura*, which have been assembled by DARLINGTON (1934a), constitute the primary cytological data upon which the reciprocal chiasmata hypothesis rests. The actually visible mechanical relations and behavior of the sex chromosome bivalents described by DARLINGTON have been corroborated by DOBZHANSKY (1934) and STURTEVANT and DOBZHANSKY (1936) for *Drosophila pseudoobscura* itself, and seemingly iden-

tical properties have been discovered in the autosomes of Melophagus (COOPER 1941) and autosomes and sex chromosomes of Olfersia (present paper). The merits of DOBZHANSKY's claim (*vide* COOPER 1941) that he has cytological proof of reciprocal chiasmata between X and Y in *Drosophila duncani* can be assessed only after publication of the data.

The cytological evidence for reciprocal chiasmata in the sex chromosomes of *Drosophila pseudoobscura* are catalogued by DARLINGTON (1934a, 1937, 1939b) much as follows, and the quotations are from his work. Each item of this list is accompanied by my judgment of its significance. All the points he gives involve directly or indirectly a comparison of sex chromosomes with autosomes. It is by no means clear that his contrast of the sex chromosome bivalent with the autosomes may legitimately be employed to argue the existence of chiasmata in the former, for DARLINGTON (1937, p. 372) points out that "*the autosome pairs . . . would be incapable of showing chiasma formation, even if they had crossed over or not*, because their four chromatids are equally attracted to one another, lying equally parallel at diakinesis" (italics mine). However, there is no difficulty in judging whether the evidence presented justifies the conclusion that chiasmata are present.

(a) *At diakinesis the autosomes are associated at their kinetochores, whereas the sex chromosomes are associated not at the kinetochores, but at interstitial loci near their kinetochores.* There is nothing here that suggests or denies the existence of chiasmata. DARLINGTON (1934b) has already committed himself to both the belief that (a) the sex chromosomes can pair at their kinetochores and (b) that crossing over may occur within the "attachment chromomere" itself. Furthermore, DARLINGTON (1934a, p. 97) states that the proximal and distal ends of the autosomal bivalents are not distinguishable at this stage.

(b) *The X and Y are only in contact over a short portion of their length and this is evidently not the whole of the homologous segment.* The cases of Olfersia (*vide ut supra*) and Melophagus (COOPER 1941) show such localized conjunction to be possible between homologous chromosomes, both interstitially and terminally, without involving chiasmata. By itself, this feature of sex chromosome conjunction is not *a priori* evidence for chiasmata.

(c) *In the restricted region of conjunction (b above) the sex chromosomes "come into closer contact than the autosomes, sometimes, and at other times rather less close—no visible connection joining them."* This observation concerning some sex chromosome bivalents which are less closely associated than the autosomes awakens the suspicion that conjunction of the sex chromosomes in *Drosophila pseudoobscura* may occur much as described in Olfersia above.[10] STURTEVANT and DOBZHANSKY (1936), in considering the action of "sex ratio" in *Drosophila pseudoobscura*, point out that the failure of X and Y to form a bivalent may be owing to persistence of an initially separate state of the two chromosomes. DARLINGTON (1934a, pp. 98, 100, 109) states that at

[10] KOLLER and TOWNSON (1933, p. 134) remark that X and Y are seen to pair during condensation in some spermatocytes of *D. pseudoobscura*. Their figures are too poor to give weight to this observation.

diakinesis there are some sex chromosome pairs between the two chromosomes of which *no* connection is visible. This he interprets (p. 109) as a "lateral chiasma" which is not to be expected to give a visible connection between X and Y. Such a chiasma could arise only by terminalization, but DARLINGTON has shown that there is no repulsion between sex chromosome kinetochores in diakinesis. The required mechanism of terminalization is therefore in abeyance at this stage. Perhaps the observed loose association of X and Y may be accounted for by a chiasma interpretation. Nevertheless it cannot be marshalled as evidence demanding the occurrence of chiasma in the sex bivalent.

(d) *The connection (locus of conjunction) may be on either side of the kinetochore, but is never on both sides. "It therefore shows the variation and the interference characteristics of chiasma formation."* If homologous loci exist in both arms of Y, but in only one arm of X, this observation should follow whether chiasmata are the mechanism of conjunction or not. This observation cannot be considered as proof, or indeed evidence, for chiasmata between the sex chromosomes. Similar observations are described above for the sex chromosomes of Olfersia where chiasmata are clearly not involved.

(e) *"At metaphase a state of tension develops between the spindle attachments and the point of association, and the chromosomes do not separate gradually at this point, but suddenly."* This observation affords no proof of chiasmata. Tension develops between the spindle attachments of the autosomes and their paired regions as well (see figures in DARLINGTON 1934a, DOBZHANSKY 1934, STURTEVANT and DOBZHANSKY 1936, etc.), as DOBZHANSKY (1934) has stated. Furthermore the supposed suddenness of separation may be questioned. It should be noted that the sex bivalent has only a very short length to disjoin relative to that of the autosomes, and perhaps only for this reason appears to disjoin suddenly. Lastly, these observations are paralleled by those on the autosomes of Melophagus and all large chromosomes of Olfersia (this paper) where an assumption of chiasmata is unwarranted.

(f) *The X and Y are unchanged when they separate at anaphase (that is, they preserve their respective identities).* This is likewise a characteristic of the autosomal bivalent of male Drosophila between the respective chromosomes of which no chiasmata need be inferred. In fact any mode of conjunction not involving crossing over will give this result, whereas only one type of chiasma production, namely reciprocal pairs of chiasmata, can account for these results. While explicable on the latter hypothesis, it cannot be considered as evidence which requires this hypothesis.

(g) *"The lack of time coordination between spindle, autosomes, and sex chromosomes* (that is, the sex chromosomes may precede or lag—K.W.C.) *found also in D. melanogaster, is an indication that two independent processes of development are at work in the same nucleus."* This certainly cannot be taken as evidence for the occurrence of reciprocal chiasmata in the sex chromosomes. Were the argument pursued to its logical end, each chromosome would have to be considered conjoined by a unique mechanism, for all the bivalents appear to vary in their timing of anaphasic disjunction.

(h) "*The sex chromosomes fail to pair in a proportion of the cases, although the autosomes are regularly paired.*" Failure of any conjunctive mechanism should give rise to non-disjunction. Hence this is not evidence for the occurrence of chiasmata between X and Y. Furthermore the figures (DARLINGTON 1934a, 28 *et seq.*) in support of this statement may be given alternative interpretations. For example, none of these figures shows both sex chromosomes to have gone to the same pole, and figure 29 of supposed non-disjunction compares very favorably with figure 22 purporting to show precocity of the sex chromosomes.

There are additional cytological considerations (see pp. 552–554) which make the reciprocal chiasmata hypothesis difficult to maintain and require assumptions (3c–e) above. Furthermore, the characteristic loop of the reciprocal chiasmata and the clear rift between sister chromatids (DARLINGTON 1937, fig. 115, C3; fig. 36B) have never been found in the case of fly sex chromosome bivalents. It may be argued that these criteria are below the limits of resolution, in which event the hypothesis cannot be put to direct proof.

Thus it may be concluded that there is no genetic or cytological evidence known which requires the assumption of reciprocal chiasmata between X and Y in male Drosophila or the male of any other fly. Indeed some of the evidence available is directly opposed to such a hypothesis.

DISCUSSION

Comparison of meiotic conjunction of the chromosomes of Olfersia, the autosomes of Melophagus, and the sex chromosomes of Drosophila suggests a common conjunctive mechanism which does not directly involve any elements of either chiasmatype theory or the chiasma hypothesis of metaphase pairing. But until further data are accumulated, little more than a descriptive analysis can be given.

The chromosomes under discussion behave as though they are provided with one or more relatively short "conjunctive segments" which are responsible for cohesion of homologous chromosomes in bivalents and hence necessary for segregation at meiosis. These segments may also be responsible for the initial coming together of homologs which inaugurates bivalent formation at meiosis, but this does not necessarily follow from what is known. In Melophagus and Olfersia the conjunctive segments appear to act as loci from which the factor(s) customarily described as "forces of somatic pairing" emanate and operate during ordinary mitotic divisions. It is not known whether such strict localization of somatic pairing forces occurs also in the chromosomes of Drosophila,[11] but it is clear that two interstitial regions in the Y and at least one in the X of *D. pseudoobscura* males behave as though they alone possess a capacity for cohering at meiosis. On the other hand the autosomes of Drosophila appear to succeed in conjoining as bivalents at meiosis without such an apparently intimate contact as that provided by the localized conjunctive segment in the cases under discussion. Whatever the mechanism may be which holds these

[11] See Addendum, page 568.

autosomes together, the simplest interpretation seems to be that the same mechanism operates in all the cases considered, but that the means of actual cohesion is restricted and localized by some regional specialization of those chromosomes which are endowed with a conjunctive locus. Now if this is so, the relatively small segment must be capable of producing a cohesion of homologs fully as effective as that provided by the entire length of chromosome which participates in the conjunctive effort of an autosome of the Drosophila male. Such a consideration perhaps accounts for the fact that homologs appear to be actually locally united when they are paired by means of conjunctive segments.

Such restricted loci which do not cross over but which do bind homologs together as bivalents in the male nicely account for such genetical and cytological properties of the sex chromosomes of Drosophila as have been discussed in the preceding section. Furthermore such a mechanism is in harmony with additional data that militate against any hypothesis involving regular reciprocal crossing over between the inert regions of X and Y. "In(1)Df*bb*" is an X chromosome of *Drosophila melanogaster* which has lost about one-third its length, and this from the inert region. In spite of the extensive loss of inert material, only about three percent non-disjunction of X^{bb-df} and Y occurs in the male (SIVERTZEV-DOBZHANSKY and DOBZHANSKY 1933). Presumably loss of most of the inert region has not resulted in considerable loss of efficiency of the conjunctive segment, although the defect in the X chromosome is of such magnitude that it should prohibit reciprocal chiasmata formation. GERSHENSON (1933) has described another bobbed-deficient X which, while giving high non-disjunction of X and Y in the male of *D. melanogaster*, does not result in purely random segregation of X and Y, there being a pronounced tendency toward regular disjunction. Here it may be supposed that the loss from the inert region includes a relatively large one from the hypothetical conjunctive segment, but such that enough remains to preclude absolutely random segregation. On the reciprocal chiasmata hypothesis disjunction should remain normal, or produce many single exchanges between X and Y, or become purely random.

As DARLINGTON (1937, p. 392) points out, the origin of the homologous inert segments remains unexplained on the basis of the reciprocal chiasmata hypothesis. If, however, the dipteran ancestral types did have their meiotic segregation conditioned by chiasmata in both male and female, then with reduction of crossing over in the male there must have been selection for efficient mechanisms capable of supplanting crossing over in the male, or for rigid localization of simple crossing over. Both appear to have evolved in the Diptera, but a non-chiasmate mechanism seems to be the dominant if not the only mode of conjunction in male muscoids and Pupipara. Such a mechanism results in maintaining the Y in a continually heterozygous state. This, as MULLER (1914, 1918; MULLER and PAINTER 1932) first pointed out, means that Y is largely insulated from the action of natural selection and may accumulate degenerative mutations to inertness.

If, as is suspected, the "conjunctive segment" proves to be a non-genic

organelle of the chromosome, like the kinetochore complex, matrix, nucleolus organizer, etc., then the name "collochore" is suggested for it. No implication as to the mechanism of action is to be considered implied by the literal meaning of the Greek compound. As appears to be the case for both the kinetochore and nucleolus organizer, the collochore seems to be able to function after it has been fractured.

SUMMARY AND CONCLUSIONS

In the male of the fly *Olfersia bisulcata* the diploid number of chromosomes is eight. The haploid chromosome set comprises a dot-like, a rod-like, and a V-shaped autosome, as well as either a large submedian X′ or a smaller nearly median Y′.

At the spermatogonial mid-prophase somatic pairing is restricted to a distal region in the rod-shaped autosomes, and to one terminal and two interstitial regions in the V-shaped autosomes; at points other than these "conjunctive segments" the homologs are either indifferent to or repel one another. A remarkable feature of the somatic pairing of the V-shaped autosomes is the fact that they twist about one another only at their interstitial pairing loci.

Although the sex chromosomes show no tendency for somatic pairing at gonial prophase, they as regularly lie adjacent to one another at metaphase as do homologous autosomes (that is, nearly always).

At diplotene and early diakinesis the bivalent configurations simulate their corresponding somatically paired homologs of gonial prophase. Thus the rod-shaped autosomes are conjoined distally only, and the V-shaped autosomes are conjoined at one terminal and two interstitial loci. The sex chromosomes are totally separate from each other.

At diplotene the homologs of the V-shaped autosome bivalent are twisted about one another at the interstitial pairing segments. Accordingly this bivalent superficially appears to possess at least three chiasmata. With approach to metaphase I, however, the twists are largely undone and the disjunction figures—coupled with antecedent phenomena—show this bivalent as well as the others to be devoid of chiasmata.

The sex chromosomes, which may be a nuclear diameter apart at diplotene, come together and ankylose in a short interstitial region before the close of diakinesis. Although the resulting bivalent behaves and appears thereafter identical in structure with the sex chromosome bivalent of the male *Drosophila pseudoobscura*, clearly it cannot be provided with chiasmata unless, as is very improbable, condensed chromosomes can undergo crossing over.

The autosomal and sex chromosomal bivalents thus owe their paired condition at first metaphase not to the formation of chiasmata, but to the possession of short regions of their lengths ("conjunctive segments") to which appear restricted the means of cohesion as bivalents. Whether these regions are truly anatomical specializations of the chromosomes is undecided.

The phenomena described above throw a wholly new light on the observations of the sex chromosome bivalent of male *Drosophila pseudoobscura* where

reciprocal chiasmata are said by DARLINGTON to be the sole mode of conjunction at first meiotic metaphase. The reciprocal chiasmata hypothesis is accordingly precisely formulated and analysed. Detailed consideration of the cytological and genetical evidence available for Drosophila leads to the conclusion that the reciprocal chiasmata hypothesis is unnecessarily involved and neither required nor supported by the relevant data.

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ADDENDUM

Since this went to press, Philip *et al.* (Nature **154**: 260–262, 1944) state that, unlike the case of the autosomes, somatic pairing of X and Y occurs only at the proximal ends in *Drosophila subobscura*.

INHERITANCE OF BULB COLOR IN THE ONION

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IN THE onion the color of the bulb ranges from white to dark red and dark yellow, a great assortment of shades of red and yellow being known. The attractiveness of a variety depends to a large degree upon the bulb color. Furthermore, colored bulbs are highly resistant to the onion smudge organism, *Colletotrichum circinans* (Berk.) Vogl., whereas white bulbs are susceptible. For these reasons it is important to understand the mode of inheritance of different colors.

Genetic studies in this subject have been published, but in this paper data from additional crosses are presented which necessitate a modification of the conclusions drawn from this earlier work.

REVIEW OF LITERATURE

TSCHERMAK (1916) found that dark yellow and red bulb colors are dominant to white, with a complex segregation in the F_2. MEUNISSIER (1918) reported that sometimes yellow is dominant over red and over white, although a recessive yellow also occurs.

RIEMAN(1931) has made a more detailed study of the inheritance of bulb color in the onion. He postulated a series of multiple alleles— namely, W a gene for red pigment, W^v a gene for yellow pigment, and w a gene for white. W is dominant over both W^v and w. In addition, there are the genes I and i, inherited independently of this allelic series. I is incompletely dominant over i and inhibits both red and yellow so that all plants homozygous for I are white. Both red and yellow bulbs lack the inhibitor I, the homozygous red onions being $ii\ WW$ and the homozygous yellow being $ii\ W^v\ W^v$.

MATERIALS AND METHODS

To determine the mode of inheritance of red, yellow, and white bulb color crosses were made between a large number of commercial varieties and inbred strains. Flies were used to facilitate crossing, as described by JONES and EMSWELLER (1934). Several populations were grown in the field at Milpitas, Calif., in 1934, while the second author was a member of the staff of the College of Agriculture, UNIVERSITY OF CALIFORNIA, but most were grown since 1936 in the greenhouses at the Plant Industry Station, Beltsville, Md. F_1, F_2, F_3, and backcross and backcross selfed populations have been examined. For convenience in summarizing, comparable populations have been pooled in the tables.

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The goodness-of-fit to Mendelian ratios was calculated by the chi-square method.

EXPERIMENTAL RESULTS

In reciprocal crosses between red and yellow varieties the F1 bulbs were red, showing that red is dominant over yellow (table 1). The ratios obtained were in close agreement with the expected ratios of 3 red: 1 yellow for the F2 and 1 red: 1 yellow for the backcross populations. The genes differentiating red pigment from yellow were designated by RIEMAN (1931) as W–W^v.

TABLE 1

Inheritance of bulb color in the onion in crosses involving the C and R genes.

PEDIGREE	GENOTYPE	GENERATION	OBSERVED			EXPECTED RATIO	P
			RED	YELLOW	WHITE		
Red×yellow	*ii CC RR*×*ii CC rr*	F1	All				
Yellow×red	*ii CC rr*×*ii CC RR*	F1	All				
F1 red selfed	*ii CC Rr* selfed	F2	2,353	756		3:1	0.40
F1 red×yellow	*ii CC Rr*×*ii CC rr*	B1	214	226		1:1	.58
Yellow×white	*ii CC rr*×*ii cc rr*	F1		All			
F1 yellow selfed	*ii Cc rr* selfed	F2		638	216	3:1	.85
White×F1 yellow	*ii cc rr*×*ii Cc rr*	B1		14	14	1:1	1.00
Yellow selfed	*ii Cc rr* selfed	B1(X)		94	36	3:1	.48
Red×white	*ii CC RR*×*ii cc RR*	F1	All				
F1 red selfed	*ii Cc RR* selfed	F2	421		154	3:1	.33
F2 red selfed	*ii Cc RR* selfed	F3	121		43	3:1	.72
Red×F1 red	*ii CC RR*×*ii Cc RR*	B1	All				
F1 red×white	*ii Cc RR*×*ii cc RR*	B1	86		69	1:1	.18
Yellow×white	*ii CC rr*×*ii cc RR*	F1	All				
White×yellow	*ii cc RR*×*ii CC rr*	F1	All				
F1 red selfed	*ii Cc Rr* selfed	F2	1,019	375	482	9:3:4	.21
Yellow×white	*ii CC rr*×*ii cc Rr*	—	15	19		1:1	.49
Red×white	*ii Cc Rr*×*ii cc Rr*	—	59	25	80	3:1:4	.57

In certain crosses between yellow and white plants the F1 bulbs were yellow, the F2 produced a ratio of 3 yellow: 1 white, and the backcross produced 1 yellow: 1 white (table 1). When yellow-bulbed plants from the backcross were selfed, they also produced 3 yellow: 1 white. This indicates that in these particular crosses white bulb color behaves as a simple monogenic recessive to yellow. RIEMAN (1931) used the gene symbols W^v and w to differentiate yellow from recessive white.

Some crosses between red and white produced red F1 plants. On selfing they produced F2 bulbs in the ratio 3 red: 1 white. When backcrossed to the red parent, the progeny were all red, whereas when backcrossed to the white parental strain, the progeny were 1 red: 1 white. According to RIEMAN's nomenclature, the genes involved in this case are W and w.

Several yellow×white crosses were also obtained in which all the F1 plants were red. When the F1 plants were selfed, a satisfactory fit to the dihybrid ratio 9 red:3 yellow:4 white was obtained in the F2 (table 1). These F1 and F2 results cannot be accounted for by RIEMAN's multiple allele hypothesis. Consequently, it is postulated that a basic color factor *C* is involved in the above red × white and yellow × white crosses, and the genes differentiating red color from yellow in the presence of *C* have been designated *R-r*. Recessive white plants *cc* may carry either *R* or *r*.

A different yellow × white cross gave red and yellow plants in equal numbers. This ratio of 1 red:1 yellow can be accounted for by assuming that the white plant involved in the cross was heterozygous for the *R* factor, the parental genotypes being *CC rr* (yellow)×*cc Rr* (white).

An F_1 red plant known to have the genetic constitution *Cc Rr* was crossed with a white plant belonging to the genotype *cc Rr*. A satisfactory fit was obtained to the ratio 3 red:1 yellow:4 white (table 1).

In some crosses between red and white the F1 plants are not as red as in the crosses previously described but are intermediate in color. This behavior is found in crosses involving dominant white gene *I*, as previously described by RIEMAN (1931). This gene is incompletely dominant so that some color develops in the heterozygous *Ii* plants, the amount of pigmentation depending apparently on what other color genes are carried by the plant. It was not possible to distinguish with certainty *Ii CC RR* from *Ii CC rr*. All plants involved in the crosses shown in table 1 belong to the genotype *ii*. Data from crosses involving the inhibitor *I* are shown in table 2. In the cross *ii CC RR*×*II CC RR* the F1 plants are intermediate and the F2 segregate in the ratio 1 white:2 intermediate:1 red. Frequently it is difficult to differentiate between the white and intermediate classes, so that the ratio becomes 3 white and intermediate:1 red. When tested in the F_3 generation, all white and red F2 plants bred true; all intermediate plants again segregated. When the F1 intermediate was backcrossed to dominant white, a satisfactory fit to the expected ratio of 1 white:1 intermediate was obtained. A similar ratio was obtained from the reciprocal backcross. Some of the intermediate plants from the backcross were selfed. As shown in table 2, they gave an extremely poor fit to the ratio 1 white:2 intermediate:1 red, but this was probably the result of difficulty in distinguishing between whites and intermediates, since a satisfactory fit was obtained when the white and intermediate classes were grouped together to give the ratio 3 white and intermediate:1 red. When an intermediate plant from a cross between dominant white and red (*II CC rr*×*ii CC RR*) was backcrossed with the red parent, a satisfactory fit to the expected ratio of 1 intermediate :1 red was obtained.

Intermediate plants belonging to the genotype *Ii CC rr*, obtained from the cross *II CC rr* (white)×*ii CC rr* (yellow), cannot always be distinguished with certainty from pure white. When the whites and intermediates were grouped together in the F_2, a satisfactory fit was obtained to the ratio 3 white and intermediate: 1 yellow (table 2). The backcross *Ii CC rr* (intermediate)

×*ii CC rr* (yellow) also gave a satisfactory fit to the expected ratio 1 intermediate: 1 yellow. A satisfactory fit to the ratio 3 white and intermediate: 1 yellow was also obtained by selfing an intermediate plant selected from a backcross population.

TABLE 2

Inheritance of bulb color in the onion in crosses involving the I gene.

PEDIGREE	GENOTYPE	GENERATION	OBSERVED				EXPECTED RATIO	P
			WHITE	INTERMEDIATE	RED	YELLOW		
Red×white	*ii CC RR*×*II CC RR*	F1		All			1:0	
F1 intermediate selfed	*Ii CC RR* selfed	F2	63	156	58		1:2:1	0.10
F1 intermediate selfed	*Ii CC RR* selfed	F2	35*		6		3:1	.13
F2 intermediate selfed	*Ii CC RR* selfed	F3	339	727	321		1:2:1	.16
F2 intermediate selfed	*Ii CC RR* selfed	F3	107*		41		3:1	.46
F1 intermediate ×white	*Ii CC RR*×*II CC RR*	B1	333	363			1:1	.26
White×F1 intermediate	*II CC RR*×*Ii CC RR*	B1	31	32			1:1	.90
Intermediate selfed	*Ii CC RR* selfed	B1(X)	136 (564*)	428	187 (187)		1:2:1 (3:1)	.01–(.95)
Intermediate ×red	*Ii CC Rr*×*ii CC RR*	B1		297	265		1:1	.18
F1 intermediate selfed	*Ii CC rr* selfed	F2	737*			252	3:1	.73
Intermediate ×yellow	*Ii CC rr*×*ii CC rr*	B1		92		72	1:1	.12
Intermediate selfed	*Ii CC rr* selfed	B1(X)	10*			5	3:1	.47

* Includes intermediates, which were not distinguished from whites.

F2 populations segregating for both the *I-i* and *R-r* genes were obtained from both red×white (*ii CC RR*×*II CC rr*) and white×yellow (*II CC RR*×*ii CC rr*) crosses. These populations gave a satisfactory fit to the ratio 12 white and intermediate: 3 red: 1 yellow (table 3). From selfing the F1 intermediates from the cross *II cc RR* (white)×*ii CC rr* (yellow) a satisfactory fit to the trihybrid ratio 52 white and intermediate: 9 red: 3 yellow was obtained (table 3). No populations segregating for only the *I-i* and *C-c* genes were tested. However, if the red and yellow classes are pooled in the foregoing case, a satisfactory fit to the expected ratio 13 white and intermediate: 3 colored is obtained, the P value being 0.55. The backcross *Ii Cc* (intermediate)×*II cc* (white) gave a satisfactory fit to the ratio 3 white: 1 intermediate (table 3).

TABLE 3

Inheritance of bulb color in the onion in crosses involving the I, C, and R genes.

PEDIGREE	GENOTYPE	GENERATION	OBSERVED				EXPECTED RATIO	P
			WHITE	INTERMEDIATE	RED	YELLOW		
F1 intermediate selfed (from red×white)	*Ii CC Rr* selfed	F2	94*		19	11	12:3:1	0.35
F1 intermediate selfed (from white×yellow)	*Ii CC Rr* selfed	F2	2,921*		701	259	12:3:1	.35
F1 intermediate selfed (from white×yellow)	*Ii Cc Rr* selfed	F2	338*		52	20	52:9:3	.72
F1 intermediate×white	*Ii Cc*†×*II cc*†	B1	33	14			3:1	.46

* Includes intermediates, which were not distinguished from whites.

† These plants may have carried either *R* or *r*.

In an F2 population segregating for both white and colored bulbs, an association was found between the color of the bulb and its weight in grams. During a study of the effect of length of photoperiod on bulbing, an F2 population from the cross Southport White Globe (*II CC RR*)×Early Grano (*ii CC rr*) was grown in the greenhouse under 13-, 14-, and 15-hour photoperiods. The bulbs were classified according to color into three groups: (1) white, (2) intermediate, and (3) red and yellow. The white bulbs belonged to the genotype *II*; the intermediate bulbs to *Ii*, the *I* factor being only incompletely dominant over red and yellow; and the red bulbs to *ii*. It was difficult to distinguish always between the *II* and *Ii* genotypes, so an occasional bulb may have been erroneously classified. The individual bulbs were weighed in grams, and it was found (table 4) that for each photoperiod the white bulbs (*II*) weighed less than the red and yellow ones, the difference being sufficiently great to exceed the 1 percent level of significance.

TABLE 4

Weight of white and colored F2 onion bulbs from the cross Southport White Globe ×Early Grano, grown in 13-, 14-, and 15-hour photoperiods.

COLOR	GENOTYPE	13-HOUR	14-HOUR	15-HOUR
		Grams	*Grams*	*Grams*
White	*II*	43.8±2.32	39.7±1.59	36.7±1.56
Intermediate	*Ii*	50.0±1.41	43.0± .94	45.0± .80
Red and yellow	*ii*	56.1±2.66	47.6±1.59	45.4±1.52

DISCUSSION

As evidence that the genes *W*, *W*^{v}, and *w* form a series of alleles, RIEMAN showed that a simple monohybrid relationship exists between the genes *W* and

w and likewise between *W* and W^v. Unfortunately, as RIEMAN pointed out, all of his yellow×white crosses involved white plants carrying the inhibitor *I* so that the relationship between W^v and *w* was not definitely established. In this paper data are presented which show that in certain crosses, in which the inhibitor *I* is not involved, a simple monohybrid relationship exists between yellow and white. This completes the proof that the genes for red and yellow are alleles but does not necessarily establish the validity of RIEMAN's multiple allele hypothesis, since the recessive white class can be explained in another way. It may be assumed that there is a basic color factor *C*, which is essential for pigment formation, so that plants possessing *C* are colored and those with *c* are white. In the presence of *C* the dominant gene *R* produces red pigment, and the recessive *r* produces yellow. Red varieties carry the genes *i C R*, yellow varieties *i C r*, and recessive white may be either *i c R* or *i c r*. All varieties which carry the dominant inhibitor *I* are white, regardless of the presence or absence of the *C* and *R* factors.

This hypothesis fits RIEMAN's data equally well as did his multiple allele hypothesis and has the advantage of explaining certain results presented in this paper, which cannot be accounted for by the assumption of multiple alleles. All the F_1 plants from certain yellow×recessive white crosses (*ii CC rr*×*ii cc RR*) had red bulbs. Furthermore, all three color classes segregated in the F_2—namely, red, yellow, and white. Since these results are contrary to those expected on RIEMAN's hypothesis, data on the color of bulb in the onion are interpreted in this paper on the basis of three independent pairs of factors: *C-c*, *R-r*, *I-i*.

The data presented in table 4 show that there is a correlation between weight and color of bulb, the white bulbs tending to be smaller than the colored ones. This suggests that, in this particular cross, the gene *I* either has a direct effect on the size and weight of the bulb or else is genetically linked with one or more growth factors which influence the weight of the bulb. This point is of considerable practical importance and deserves further study because it will be impossible to secure large white onions carrying the *I* factor if this gene is directly responsible for the smaller bulb size. On the other hand, if the difference in size is the result of a linkage between *I* and a factor for growth, it should be possible to secure some crossing over, and to establish stocks of large-size bulbs which are also homozygous for *I*.

SUMMARY

Three pairs of genes are involved in the development of pigment in the onion bulb—namely: (1) *C-c*, a basic color factor, the dominant *C* gene being necessary for the production of any pigment. Consequently, all *cc* plants produce white bulbs. (2) *R-r*, in the presence of *C*, the dominant *R* gene is responsible for the production of red pigment; its allele *r* is responsible for yellow pigment. (3) *I-i*, an inhibiting factor *I* is partially dominant over *i*. All *II* plants produce white bulbs.

In one cross a correlation was found between color and weight of bulbs, sug-

gesting either that the gene *I* is genetically linked with one or more growth factors or that this gene is directly responsible for smaller bulb size.

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GENETICS OF PARAMECIUM BURSARIA. IV. A FOURTH VARIETY FROM RUSSIA. LETHAL CROSSES WITH AN AMERICAN VARIETY[1]

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EARLIER papers (JENNINGS 1938, 1939, 1941, 1942) have described in *Paramecium bursaria* from the United States three non-interbreeding varieties, each with a characteristic set of mating types or sex types. Variety I has four mating types which have been designated A, B, C, and D. Variety II has eight mating types, designated E, F, G, H, J, K, L, and M. Variety III includes four mating types, N, O, P, and Q. The three varieties thus include 16 diverse mating types. Under appropriate conditions animals belonging to any one mating type of a variety will conjugate with animals of all other types of that variety. But animals of the same mating type do not conjugate together; and members of any one variety do not mate with members of the other two varieties.

An important extension of our knowledge of these matters has come through study of collections of *Paramecium bursaria* from Russia. Through the kindness of PROFESSOR G. F. GAUSE, DR. T. T. CHEN in July, 1941, obtained living cultures of *Paramecium bursaria* collected near Moscow. From the cultures a considerable number of clones were obtained, which have been under study by us since the date mentioned.

The cultural, breeding, and experimental work on these clones is here presented. The cytological work will be presented separately by T. T. CHEN. It is a pleasure to acknowledge our indebtedness to PROFESSOR GAUSE for sending us this valuable material.

MATERIAL AND METHODS

On July 12, 1941, forty vials of cultures of *Paramecium bursaria* were received from Russia. The animals were sent in a salt solution consisting of 0.03 gm $Ca(NO_3)_2$, 0.01 gm KNO_3, and 0.01 gm $MgSO_4 \cdot 7H_2O$, dissolved in 500 cc of redistilled water; to this some yeast (*Tortula utilis*) had been added. Small bits of hay stalks were also included in some of the vials. In many vials, especially those containing the bits of hay, the animals died before reaching Los Angeles. (Experience has shown that when Paramecium is sent by mail or otherwise, in small vials or tubes, success is best when no organic matter is included, the animals being merely in water or an appropriate salt solution.) In other vials a number of animals, varying from one to many, survived. Thirty-seven individuals were isolated from the collection; from these, 35 successful clones were obtained. They were cultivated in a dilute lettuce in-

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fusion to which bacteria had been added, as recently described by JENNINGS (1942).

The 35 clones were designated Ru 1 to Ru 35. Clones Ru 1 to Ru 3 came from three individuals of one vial, clones Ru 4 to Ru 19 from a second vial, clones Ru 20 to Ru 34 from a third, clone Ru 35 from a fourth. All the clones from a single vial may of course originally have constituted but a single clone, so that there may have been but four distinct clones in the entire collection.

EXPERIMENTAL STUDIES

On these 35 clones from Russia four different sets of experimental breedings were carried through: (1) To determine whether the Russian clones belong to any of the three varieties known for the United States, or whether they constitute a fourth variety. (2) To discover whether different mating types exist among the Russian clones. (3) A study was made of self-differentiation and self-fertilization in one of the Russian clones, with special reference to the number of different mating types in the Russian variety. (4) A fourth set of experimental studies dealt with crosses between a Russian variety and an American variety, and their lethal results.

Relationship of Russian clones

To determine whether or not the Russian clones belong to any of the three varieties and 16 mating types known for the United States, they were tested successively with the mating types of these known varieties.

These tests were carried out in the way described by JENNINGS (1941). Clones belonging to each of the 16 known mating types were kept in a laboratory. Abundant cultures of each known type, called "testers," were at hand. To discover whether or not any of the Russian clones belong to a particular United States variety, individuals of each Russian clone must be mingled separately with at least two of the different mating type testers of that variety. If they belong to the variety in question, they will conjugate with at least one of any two mating types of that variety (since a clone of a given mating type conjugates with all types of its own variety, except the type to which it belongs).

In the actual tests, each Russian clone was tested with all four mating types of Variety I, all four of Variety III, and with four of the eight types of Variety III. There were thus twelve tests for each of the 35 Russian clones, making 420 tests in all. In these tests the Russian clones did not conjugate with any of the three United States varieties. Later, as will be set forth, conjugations occurred between one of the Russian types and four of the eight mating types of Variety II. But such conjugations resulted always in death. Thus no normal conjugations occurred between the Russian clones and any of the United States types. The Russian clones, therefore, belong to a different variety or varieties than the three known to occur in the United States.

Mating Types

To determine if in the Russian clones more than one mating type occurs, each of the 35 Russian clones was tested with every other, by mixing individ-

uals of the two clones that were under test. There were thus 595 of these tests.

Certain of the Russian clones reacted together. The three clones Ru 1, 2, and 3 did not mate with each other, but all mated with any of the 16 clones Ru 4 to Ru 19. The clones Ru 4 to Ru 19 did not mate together. Thus the clones Ru 1, 2 and 3 belong to one mating type, the clones Ru 4 to Ru 19 to a second mating type. As noted earlier, clones Ru 1, 2 and 3 came from a single one of the original vials, clones Ru 4 to Ru 19 from another one of the vials. The two mating types belong to a fourth variety of *Paramecium bursaria*, since they do not react normally with the types of any of the three varieties known from the United States. This Russian variety may be known as Variety IV.

The two mating types of this new variety may be designated R and S, R being the type to which belong clones Ru 1, 2 and 3, while S is the mating type of clones Ru 4 to Ru 19.

Members of these two mating types (that is, clones Ru 1 to Ru 19) did not react sexually with any of the 16 clones Ru 20 to Ru 35, nor did any of these latter 16 clones react together. Ru 20 to Ru 35 thus seemingly belong to a different variety from Ru 1 to Ru 19. There was a very great difference in vigor between the two sets. The clones Ru 1 to Ru 19, constituting Variety IV, are throughout puny and weak. The individuals are small in size, pale, do not multiply well, and tend to die out of cultures unless the culture fluid is frequently changed. The individuals of the other variety, clones Ru 20 to Ru 35, on the other hand, are large, deep green in color, multiply rapidly, and do not die out even when the culture fluid becomes old. The difference between the two is very striking.

Self-Differentiation and Self-Fertilization in a Russian Clone. Number of Mating Types thus Produced

It became possible to carry the genetic analysis farther through the fact that the clone Ru 4 (of mating type S of Variety IV) differentiated in February, 1942, into two types, in the way described for the United States clones by JENNINGS (1941). On February 4, DR. CHEN observed pairs in a culture of clone Ru 4. The culture was turned over to JENNINGS, who obtained from it split pairs, exemplifying the two mating types into which the clone had differentiated; also a large number of pairs. From the split pairs and pairs, clone cultures were obtained.

The clone Ru 4 which had thus differentiated belonged originally to the mating type S. The first matter to be determined was whether it had differentiated into the two types already known, R and S, or whether it had produced a third type, as may occur in Varieties 1 and 2 (as described by JENNINGS 1941). The cultures from the two types derived from Ru 4—that is, the two clones derived respectively from the two members of any split pair—were tested with each of the other 34 Ru clones. It was found in all cases that one of the two members of the split pair mates with clones of the R type (clones Ru 1, 2, 3) but not with clones of the S type (clones Ru 5 to Ru 19), while the other member mates with clones of the S type, but not with clones of the R type.

This shows that the pairs from the self-differentiation of Ru 4 are composed of one individual of type R, another of type S. No additional type has been produced by the self-differentiations of clone Ru 4.

The two types R and S produced by the differentiation of the clone Ru differ greatly. Individuals of the type R are smaller, multiply more rapidly than S, and are much more viable. The individuals of S are larger, multiply much more slowly, and are less vigorous. At any given time, therefore, the cultures of R contain many more individuals than the corresponding cultures of S. The cultures of S type gradually decrease in numbers; after some months all of the originally numerous S cultures had died out, while the R cultures were all still flourishing. It is a notable fact, however, that there were no such differences between the R and S clones that came in the collection from Russia. Both types were moderately vigorous, of about the same size, and could be kept alive without difficulty. Seemingly it is only when the two types R and S are produced by self-differentiation of a single clone that they show the differences above described.

As described by JENNINGS (1941), self-differentiation in Varieties I and II of the United States at times produces types differing in size and vigor, just as occurs in the Russian clones. In the latter case the differences between the two types produced are unusually great.

We have thus far found only two mating types (R and S) among the Russian clones; and neither of these types mates with any of the Russian clones Ru 20 to Ru 35.

To determine whether more than two mating types exist in the variety represented by the mating types R and S, another method was employed. In the other known varieties, when any two types conjugate some of the pairs may produce still other mating types of the variety (JENNINGS 1942). Thus, in Variety I, the mating of types A and B produced among the different pairs all the four types A, B, C, and D; in Variety II the mating of the types L and M produced the six types E, F, H, K, L, and M.

If, therefore, large numbers of pairs from the mating of the two types R and S, could be obtained, additional types might be secured from some of the pairs, provided that such additional types occur in this variety. Should they occur, their presence would be indicated by the fact that they mate with both types, R and S—since any type mates with all other types of the same variety.

To carry out this program, many matings were made between clones of the type R and others of the type S. Some of the clones so employed were derived from the split pairs of the clone Ru 4, these split pairs consisting of one individual of type R, one of type S. Other pairs were obtained by mating the individuals of the clone Ru 3 (type R) with some one of the clones Ru 5 to Ru 19 (all being of type S).

The securing of the necessary data was rendered difficult by the lack of vigor above mentioned in the Russian clones of Variety IV. This lack of vigor shows itself in the fact that the clones do not conjugate readily, and in a very high mortality among the ex-conjugants in cases in which conjugation occurs. Many mixtures of type R with type S yield almost no pairs. By making many

such mixtures, 224 pairs were obtained, yielding after separation 448 ex-conjugants. These ex-conjugants were cultivated separately with great care, but most of them died. Of the 448 ex-conjugant clones, all but 56 died out before as many as five fissions had occurred. The remaining 56 were cultivated in the usual way each in a Syracuse dish, but none of them was vigorous. They were tested with the testers of types R and S, from the original Russian clones. Reactions occurred in 42 of the 56 the remaining 12 did not develop sufficiently to react sexually. Of the 42, some reacted only with type R, some only with type S, none reacting with both R and S. Thus it is clear that no additional types have been produced by the crossing of the types R and S. All of the progeny are again either R or S. Thirty-two were R, ten were S.

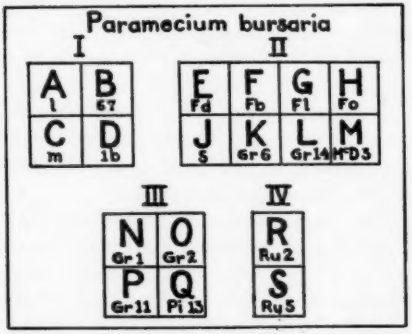

FIG. 1.—Diagram of the four known non-intercrossing varieties of *Paramecium bursaria*, with their component mating types. The eighteen different mating types are designated by the capital letters A to S. The smaller letters or figures in the lower half of each square are the designations of the clones that were first found exemplifying each type.

There is, therefore, no evidence here (nor from any other source) of the existence in the Russian Variety IV of more than the two mating types R and S. Variety IV must for the present be considered as made up of the two mating types. Figure I is a diagram of the four known non-intercrossing varieties, with their constituent mating types.

Crosses Between Certain Types of Variety IV and of Variety II Lethal Results of Such Crosses.

After the two mating types R and S were discovered in the Russian collections and these types had been extensively studied, the two were tested again with the mating types of the United States Varieties I, II, and III. There were no matings with the mating types of Varieties I and III.

But in the tests with the eight mating types of Variety II there were conjugations between the type R of the Russian collections and four of the eight mating types of Variety II. In no case did individuals of the Russian type S conjugate

with any type of Variety II. But individuals of the Russian type R formed pairs with the four mating types E, K, L, and M of Variety II. No pairs were ever formed with the other four mating types, F, G, H, and J of Variety II.

In certain of the mixtures between the Russian type and the American types many pairs were formed. In mixtures of the Russian type R and the American type E (clone "Fd") there were great numbers of pairs. Several thousand of these were fixed for cytological study by DR. T. T. CHEN.

In order to avoid possibility of error, mixtures were employed in which the individuals of one type were deep green, while those of the other type were transparent white. Thus, on August 31, 1942, a clone of type E of Variety II was used in which all the individuals were of a deep opaque green. With these were mixed individuals of type R of the Russian variety, in which all the individuals were of a transparent white. From several mixtures of this kind there were obtained, September 1 and 2, 1942, 115 pairs, in every one of which one individual was dark green (type E) while the other was transparent white (type R), the two being strongly contrasted. There can be no doubt that conjugation is in every case between one individual of type E and one of type R.

The phenomena in these cases are very different from what occurs when both mating types belong to the same variety. Only one type of the Russian variety took part in these matings, and only four of the eight types of Variety II. Normally any clone that mates with any of the types of a particular variety will mate with all types of that variety except one (that type to which it belongs). The Russian types do not belong to Variety II. Yet four of the eight types of Variety II show sufficient affinity to the Russian variety to permit conjugations to occur with one of the Russian types.

This crossing of two diverse varieties appeared of great interest. The results however turned out to be of a different kind from what was anticipated. All pairs of ex-conjugants from such crosses die without multiplication. The nature of the phenomena will be seen from description of examples:

On April 15, 1942, at 4 to 4:30 P.M., mixtures were made of clones R and S, respectively, with each of the eight types (E, F, G, H, J, K, L, M) of Variety II. There was at once some clotting in the mixtures of R with E, K, L, M, none in the other mixtures. In the morning of April 16, at about 9:30, 36 pairs were isolated from the crosses. Twenty-two were crosses of R with mating type M, ten with type E, two with type K, and two with type L. They were kept under conditions in which normal pairs thrive.

Twenty-four hours later all the pairs appeared swollen; five pairs were dead; none had separated.

Twenty-seven of the 36 pairs died without separating, most dying on the second or third day after conjugation. Nine of the pairs separated, but none of the 18 ex-conjugants divided. All died within four days after conjugation.

Again, in the period September 2 to September 4, 1942, there were isolated under favorable conditions 82 pairs of the cross of Variety II with the Russian Variety IV. The mating types crossed were E (Variety II) and R. (Variety IV). Of these 82 pairs, 60 died without separating, while 22 separated, but the 44 ex-conjugants died without dividing.

In summary, there were cultivated under favorable conditions, 118 pairs of the cross between the United States Variety II and the Russian Variety IV. All died without multiplying. Eighty-seven, or 73.2 percent, died without separating, while the remaining 31 pairs separated, but the ex-conjugants died without dividing.

The cytological conditions underlying this total mortality of the crosses are to be presented by DR. CHEN in a separate paper.

These phenomena demonstrate death resulting from incompatibility between the protoplasms of the two clones that conjugate. Each clone by itself is capable of giving rise to normal viable ex-conjugants, but when the two are brought together death results.

A Fifth Variety Among the Russian Clones?

As mentioned earlier, the 16 Russian clones denominated Ru 20 to Ru 35 did not conjugate with any of the clones of the mating types R and S of Variety IV, nor with any clones of the three United States varieties. Also there was no conjugation between members of the different clones Ru 20 to Ru 35.

It seems improbable that these clones Ru 20 to Ru 35 were immature, since they were cultivated in our laboratory for more than a year, during which time they would be expected to have become mature. Their lack of reaction indicates rather that they represent a fifth variety of *Paramecium bursaria*; a variety that does not conjugate with members of the varieties I to IV, and that all the 16 clones are of the same mating type, since they do not react together.

The members of this fifth variety, if such it is, are large, vigorous and strong-growing; they multiply rapidly. In these respects they are strongly contrasted with such members of Variety IV as we have had in the laboratory.

ISOLATING MECHANISMS

The species *Paramecium bursaria* is made up of four (perhaps five) varieties which do not intercross. The isolating mechanisms which keep these varieties separate are of two kinds: (1) One is manifested in the reactions of the individuals. Members of the different varieties when they come in contact do not mate; they do not form clots nor unite in pairs as do members of the same variety (if they are of different mating type). The failure of the individuals of different variety to clot and cling together must be due to difference in the surface chemical reactions of the different varieties; they produce different "sex stuffs." The different mating types of any single variety also differ in their sex stuffs, but the difference here is of such a nature that the individuals adhere together. But in members of different varieties the sex stuffs differ in such a way that the individuals do not adhere. This isolating mechanism is operative in the relation of four of the eight mating types of Variety II to one of the two mating types of Variety IV.

(2) The second isolating mechanism lies in the fact that when members of different varieties do conjugate, they are physiologically incompatible in such a way that the union is fatal to both and no intervarietal crosses are produced. This method operates in cases where the first method fails—that is, in the

relation of four of the eight mating types of Variety II to one of the two mating types of Variety IV.

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THE CELLULAR EXPRESSION AND GENETICS OF TWO NEW GENES IN LEBISTES RETICULATUS

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THIS paper deals with the inheritance and phenotypic expression of two new pairs of alleles controlling color patterns in the cyprinodont fish, *Lebistes reticulatus*, commonly known as the guppy. Especial attention is given to the structures and arrangements of chromatophores as affected by the various gene combinations.

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The hereditary characters of Lebistes that have previously been analyzed are chiefly color characters, and most of these show X or Y chromosome inheritance or both—that is, crossing over between X and Y. For accounts of these see WINGE (1927), BLACHER (1927, 1928), GOODRICH (1929) (a summary) WINGE and DITLEVSEN (1938). Autosomal characters hitherto described include a color character Zebrinus (WINGE 1927), Abnomis (KIRPITCHNIKOFF 1935) (publication not accessible to us), Hunchback, which was studied by R. W. HARRISON and reported in abstract by GOODRICH *et al.* (1943). A character Fredlini, briefly described by HASKINS and DRUZBA (1938) has by personal conference been shown to be identical with our Golden and will be discussed below.

MATERIALS AND METHODS

These studies were begun in 1938. Our material has been obtained chiefly from M. MATSUNO; a fish fancier, who has been very helpful in supplying us with true breeding stock. Culture methods have been described by various writers (*cf.* WINGE 1927). Fertilization is internal, and sperm may remain in the female capable of fertilizing for eight months. A single mating will often serve for a number of litters that arrive at about 28-day intervals. It is therefore necessary to use virgin females. The sexes are separated for this purpose as soon as their characteristics can be distinguished, which is usually at about 48 days after birth (GOODRICH *et al.* 1934).

GENETIC ANALYSIS

The two mutant genes here reported are designated as Blond and Golden. After mutual comparison of stocks, DR. C. P. HASKINS has kindly adopted the above terminology by which Fredlini (HASKINS and DRUZBA 1938) becomes Golden. It was also shown that another strain present in his stocks is the same as our Blond. Our results show these to be independently assorting autosomal recessives, and this is confirmed by HASKINS's experiments (per-

sonal communication). Our genetic analysis will be first presented, followed by the detailed description of the phenotypes. Here it may be stated that Blond and Golden and the double recessive Cream are all lighter in color than the dark "Wild" type.

The evidence that Blond and Golden are each simple recessives is presented in tables 1 and 2, respectively. Counts were usually made within two days after

TABLE 1

Crosses of Wild×Blond

a. Segregation in F_2 from original cross Wild×Blond
(F_1 all, 77, Wild type)

	NO. OF MATINGS	TOTAL NO. OF LITTERS	WILD	BLOND	RATIOS
Observed	7	15	224	61	3.67:1
Calculated			214	71	3:1
b. Backcross (F_1 from Wild×Blond)×Blond					
Observed	9	14	182	155	1.17:1
Caclulated			168	168	1:1

TABLE 2

Crosses of Wild×Golden

c. Segregation in F_2 from original cross Wild×Golden
(F_1 all, 257, Wild type)

	NO. OF MATINGS	TOTAL NO. OF LITTERS	WILD	GOLDEN	RATIOS
Observed	6	15	230	74	3.1:1
Calculated			228	76	3:1
d. Backcross (F_1 from Wild×Golden)×Golden					
Observed	17	18	243	207	1.12:1
Calculated			225	225	1:1

birth, as is very desirable because of the high mortality of young fish. Sex cannot be distinguished at this time. Counts, however, of survivors showed an approximately equal distribution of sexes in each phenotype. For example, in the case of two litters raised to maturity from among those included in table 1b, from an original total of 53 born 41 survived, which were distributed as follows: 9 ♂, 12 ♀, Wild type; and 9 ♂, 11 ♀, Blond. In the case of the experiments on Blond progeny, tests by crossing with Blond stock were made to determine the existence of homozygous and heterozygous individuals in the wild type F_2. In five cases the fish was shown to be heterozygous, and in two homozygous. We have in no counts of survivors found an indication of sex linkage. Both the Blond and Golden stocks breed true. Our symbols for the

two pairs of alleles are B=dominant to Blond, b=Blond; G=dominant to Golden; g=Golden.

TABLE 3

Crosses of Wild×Golden

Segregation in F_2 from original cross Blond×Golden (F_1 all wild)

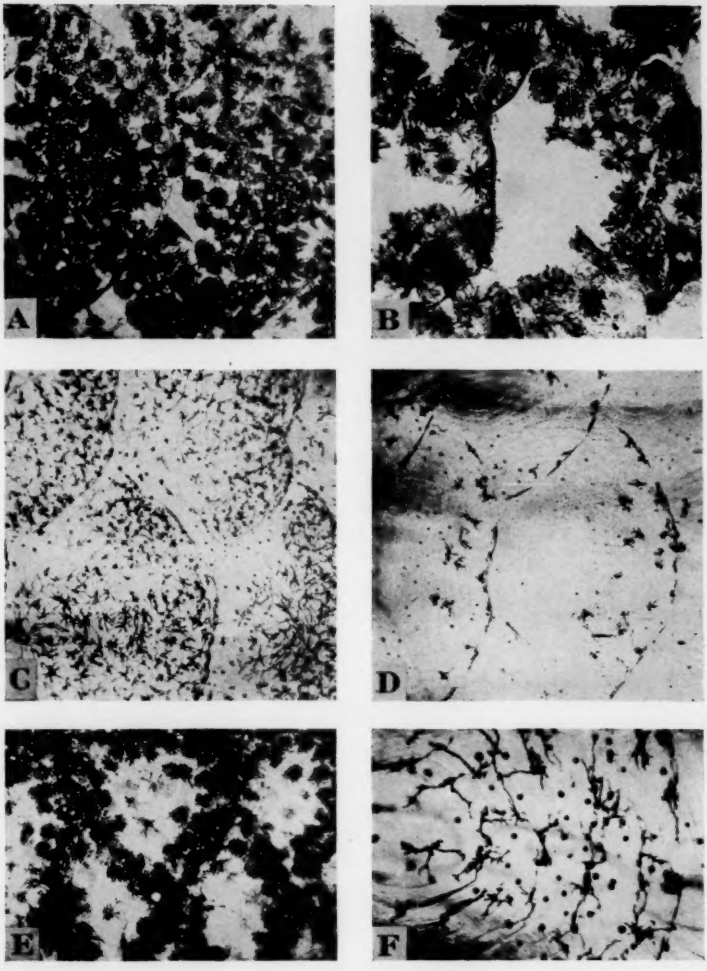
MATING NO.	WILD	BLOND	GOLDEN	CREAM	NO. OF LITTERS
122	27	9	11	1	3
123	30	10	9	3	6
124	4	3	2	1	1
126	43	19	14	3	6
133	48	13	13	4	5
135	43	16	9	7	8
136	16	6	5	1	3
137	16	4	8	1	3
138	65	18	19	4	7
139	19	10	6	2	5
141	23	13	16	4	3
142	14	8	8	4	4
143	16	3	4	1	5
144	14	5	6	2	3
Observed totals	378	137	130	38	62
Calculated distribution	384.3	128.1	128.1	42.7	

Blond and Golden

These two types were crossed, and the F_1 were all wild. The F_2 in each of 14 matings (62 litters) (table 3) produced Wild, Blond, Golden, and a new type, Cream, which gave a total of 378 Wild, 137 Blond, 130 Golden, and 38 Cream, or approximately the 9:3:3:1 ratio. One mating not included in the above total (our no. 125) gave all Wild (total of 9). While it is possible that this was due either to chance variation of the mendelian ratio or to an exceptional case requiring explanation, it seems more probable to us that it is an error in our mating records. F_1 wild type from the original cross have also recently been mated with Cream (the double recessive), and although numbers are as yet small, they confirm our interpretation. Two matings (three litters) have given total of 8 Wild, 11 Blond, 12 Golden, 8 Cream, which appears to be a first approximation of the expected 1:1:1:1 ratio. From these combined results we conclude that the two characters Blond and Golden are controlled by two independently assorting autosomal genes. In work as yet unpublished except in abstract (GOODRICH *et al.* 1943) R. W. HARRISON has shown that another gene, Hunchback, is autosomal and is also not linked to either Blond or Golden.

Cellular Analysis of Phenotypes

Our chief interest in this study lies in the comparison of the effect produced by the various gene combinations on cells and cell arrangements. The cells



EXPLANATION OF PLATE I

A–D Photomicrographs of cell pattern of the four phenotypes taken on dorsal midline slightly anterior to dorsal fin. ×43.

A, Wild; B, Golden; C, Blond; D Cream.

(C and D show clearly both dendritic and punctate melanophores).

E From side of body of Wild to show diamond pattern. ×39.

F From dorsal aspect of punctate type to show punctate and dendritic melanophores. ×80.

studied have been chromatophores—chiefly melanophores. Of these we recognize the following types or categories: (1) The dendritic melanophores (fig. 1 A–D). These are exceedingly irregular in form, and much variation is found. Most frequently there are two or three irregular processes proceeding from a relatively small cell body. They are practically always located in the dermis superficial to the scale and frequently may be lined up at the edge of a scale. (2) The corolla melanophores (fig. 1, E–F). These are so named because, in their configuration, they often resemble a composite flower with petal-like processes proceeding from a disc-like center (SUMNER and WELLS 1933). These are located in the dermis, beneath the scales. (3) The punctate melanophores (fig. 1, G–H). We believe these to be homologous with the corolla type for reasons mentioned later. They are small rounded cells, occasionally extending short processes. (4) The xanthophores. These are exceedingly delicate in outline and difficult to study.

The following are descriptions of the various phenotypes based on the appearance of the females. The female is considerably larger than the male and is uniformly colored, in contrast to the varied color patterns of the male. The distinctive color patterns of the male are due to sex-linked or Y chromosome genes (see WINGE 1927) and do not appear in the female except in cases of sex reversion, but the ground pattern of the male is essentially similar to that of the female. Our description is of the female pattern, or ground pattern, and is based chiefly on the dorsal aspect shortly anterior and posterior to the dorsal fin. There seems to be complete dominance. Variation in the heterozygote has not been observed. The Wild type (*B G*) female macroscopically is of a gray-brown color and is characterized by a diamond-shaped pattern (Plate I, E) within the meshes of which may be seen other somewhat more sparsely and irregularly distributed melanophores. On the dorsal aspect the coverage by melanophores is more uniform and the diamond pattern only dimly visible (Plate I, A). The diamond pattern is formed of the corolla melanophores (fig. 1, E), varying from 42μ to 119μ in diameter when expanded, and are located at the edges of dermal pockets in which the scales are inserted. From this it follows that they are normally observed on the body through the next anterior overlying scale. The irregularly distributed melanophores are of two types—the corolla type, deeply located beneath the scales, and the dendritic form (fig. 1, A) in the superficial dermis. Both types of cells are present at birth. Counts were made of numbers of melanophores visible in a measured area (0.9 sq mm) on various regions of the body (table 4), which gives an average of 120.1 per 0.9 sq mm.

The Golden (*B g*) female exhibits a more clear-cut but also more variable pattern than any of the other types. The difference is due to the combination of two factors: first, the larger size of both dendritic and corolla type melanophores (fig. 1, B, F), the latter varying from 70μ to 210μ in diameter; secondly, the melanophores are more definitely concentrated in the underlying diamond pattern and also above this at the edges of the scales, producing a superimimposed scalloped design which tends to accentuate the underlying diamond network. The interstices of the mesh are often clear of melanophores (Plate I,

B). The pattern may vary considerably in different parts of the body and in different individuals. This is especially noticeable in half-grown fish and gives a coarsely mottled appearance. In this respect the Golden type resembles certain conditions in the goldfish (GOODRICH and HANSEN 1931; GOODRICH and ANDERSON 1939). Neither type of melanophore is present at birth but usually appears during the first week thereafter. This is subject to considerable variation. Counts show only about 50 percent as many melanophores per unit area as in the Wild and Blond (table 4).

TABLE 4

Counts of melanophores per measured area (0.9 sq mm) in each of three regions of body in the four phenotypes.

LENGTH IN MM OF FISH TO CAUDAL PEDUNCLE																AVER.
		WILD *B G*														
		28	26	23	22	29	26	26	22	23	26	25	24	22	22	
Region*	A	142	121	157	137	77	97	116	101	76	130	79	131	112	144	115.7
	B	163	147	175	155	102	100	111	128	91	131	97	128	112	126	126.1
	C	181	151	147	139	80	91	118	103	86	109	94	122	113	124	118.4
																120.1
		BLOND *b G*														
Length		27	28	27	27	32	29	26	21	31	28	26	24	25	21	
Region*	A	116	140	111	152	89	65	125	140	149	86	155	112	106	109	118.2
	B	120	123	107	135	122	122	70	117	117	101	161	115	121	124	118.2
	C	143	158	115	166	111	97	86	118	150	120	142	139	106	139	127.8
																121.4
		GOLDEN *B g*														
Length		22	29	25	23	24	22	22	25	28	27	26	29	27	27	
Region*	A	70	64	75	69	32	49	58	57	32	61	38	75	60	79	57.7
	B	86	75	81	72	65	45	51	69	58	61	52	63	61	51	62.1
	C	84	91	65	64	42	40	51	46	52	50	48	64	65	61	58.7
																59.5
		†CREAM *b g*														
Length		25	30	23	20	29	32	27	26	24	30	29	31	22	23	
Region*	A	7	33	20	15	1	1	2	1	0	0	0	0	4	0	6.0
	B	14	27	14	8	0	1	2	1	0	0	0	0	0	0	4.8
	C	0	9	13	1	0	0	1	0	0	0	0	0	0	0	1.7
																4.2

* The regions in which measurements were made were all dorsal to the lateral line and as follows: A, posterior to operculum; B, dorsal to anus; C, 3 mm anterior to caudal peduncle.

† Counts on Cream were inadvertently made using a different magnification from other counts and the error not discovered until after death of fish used. As no other fullgrown fish were available, corrected counts based on comparative areas and corrected to the nearest whole number appear in table, except that the last two counts were made on two fish which have since then matured. For comment on variability in Cream see text.

The Blond (*b G*) female is of a uniform yellow shade. The melanophores are not visible macroscopically. The corolla type cells of the Wild are replaced by much smaller punctate cells (fig. 1, G; Plate I, C, F). These are found both in the diamond pattern beneath the scales and deeply located in the interstices of the mesh, a distribution identical with the corolla type melanophores in the Wild and Golden types. These cells as usually observed are concentrated, and only rarely and then usually only by special treatment (see below) have any of them been seen in the expanded or dispersed condition. Then they form one or two blunt short processes (fig. 1, G). The punctate cells vary from 7μ to 14μ concentrated and about twice this when dispersed. In addition there is the usual complement of superficial dendritic cells (fig. 1, C) but these average

TABLE 5

	WILD	GOLDEN	BLOND	CREAM
Genotype	*B G*	*B g*	*b G*	*b g*
Melanophores per unit area 0.9 sq mm	120.1	59.5	121.4	4.2
Diameter of corolla and punctate cells expanded	Corolla 42μ–119μ	Corolla 70μ–210μ	Punctate 7μ–14μ	Punctate 7μ–14μ

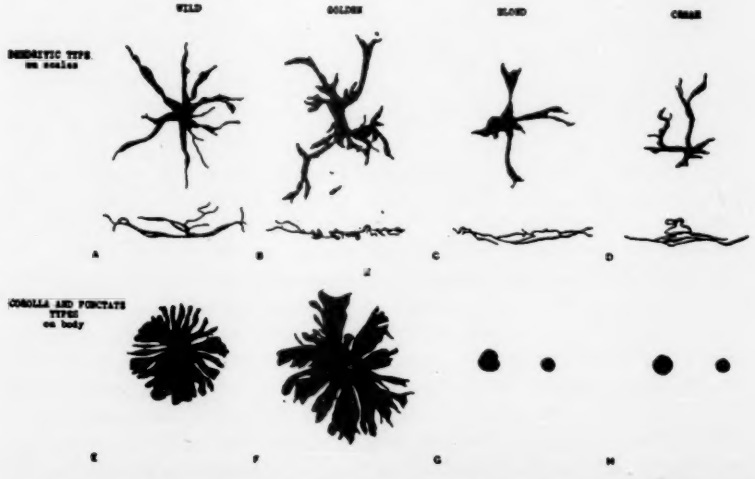


FIG. 1.—Forms of melanophores. Magnification same in all types, ×450. A–D Dendritic type: upper row, as they appear on central part of scale; lower row, at edge of scale. E–F Corolla type. G–H Punctate type. A E from Wild, B F from Golden, C G from Blond, D H from Cream.

smaller than those in the Wild or Golden types. The cell counts show a number comparable with that of the Wild form, the average per unit area being 118.1 (table 4). Both types of cells are usually present at birth. The xanthophores are fainter and probably smaller than those in the Wild.

The Cream female (*b g*) is a uniform warm yellow color and no chromatophores are macroscopically visible. The melanophores are most frequently few in number (Plate I, D) and sometimes are almost completely absent in a given area. They consist of small punctate cells (fig. 1, H) tending to follow the diamond pattern beneath the scales, while a few dendritic superficial cells (fig. 1, D) are located near the edges of the scales. Both types of cells may be present at birth. Recently we have found in our cultures some individuals having a larger number of cells, which, nevertheless, do not resemble Blond, since the cells are restricted to the edges of scales on the dorsal aspect of the body. Counts are given in table 4, and here the second and third listed cases are this latter type. There is also much variability in the different areas of a single fish. The Cream gives a positive reaction to the Dopa test, discussed below, indicating the presence of "colorless" melanophores and that therefore the fish may be light colored because of some disarrangement of normal metabolic processes. The Cream is not a hardy stock and is difficult to maintain in homozygous condition.

PHYSIOLOGICAL STUDIES

Dopa Reaction

Previous experiments (GOODRICH 1933) showed that *Oryzias latipes* responds positively to the 'Dopa' test, and since then it has been the practice in this laboratory to try the test on all light-colored mutants studied here, using Oryzias as a control. These have included varieties of the goldfish, *Carassius auratus*, *Betta splendens*, *Macropodus opercularis*. No other fish, however, had given a positive reaction until the test was applied to Cream. Tests on Blond and Golden were negative. In Cream, however, a few brown cells became visible having a configuration similar to other chromatophores. Dopa is 3:4 dihydroxyphenylalanine, which is considered to be a transformation product in the change from tyrosine to melanin (RAPER 1928). If cells show a darkening when tested, it has been interpreted as indicating that the necessary oxydase for the next step in melanin production is present but that the chromogen now supplied by Dopa had been absent. In our tests Oryzias was used as a control and gave the usual clear-cut reaction. The results in Cream were not so pronounced but nevertheless were sufficiently definite to reveal a few previously undetected chromatophore-like cells. The results have to be interpreted with caution, since work by FIGGE (1940) has indicated that under certain conditions of the reduction oxidation reaction many connective tissue cells may respond by melanogenesis. Here, however, the form of the cells seems to indicate that they were true melanoblasts. This then may indicate that the Cream fish is lighter colored either because the necessary chromogen is not present (GOOD-

RICH 1933) or because there does not exist the right oxidation-reduction potential for the enzyme to act (FIGGE 1940).

Chromatophore Reactions

GOODRICH and TRINKAUS (1940) reported that the melanophores in Blond apparently failed to show the usual responses to various stimuli and therefore might be considered as physiologically inert cells. These cells seemed to be unresponsive to light and dark backgrounds, denervation, intermedin, ergotamine, KCl, NaCl, to all of which the wild type melanophores readily respond by dispersion or concentration of pigment. Further tests by these and other agents have been made. It has been concluded that the dendritic superficial cells do clearly respond and that the punctate cells also show occasional reactions. These latter, however, are so small and the response so slight that they make very unfavorable material for nice observations. Many further experiments were tried, including electrical stimulation, injection of acetylcholine, etc. Since the results do not certainly show that the Blond cells are physiologically exceptional, the details of these extensive experiments are not reported here. However, it seems highly probable that the punctate cell is relatively sluggish in its reaction.

DISCUSSION

The charts (table 5, fig. 1) outline the most striking characteristics of the four phenotypes. It will be noticed that the Wild is in some respects intermediate between the Golden and Blond. The Golden has the larger melanophores, next is the Wild, and those of the Blond are the smallest. On the other hand, the melanophores are least numerous in the Golden type, there being about twice as many in the Blond and Wild. The Blond cells, especially the punctate type, are relatively sluggish in physiological responses compared with the Wild and Golden. The normal Wild type color pattern may be considered as a resultant of the interaction of the opposed tendencies of the two dominant genes. The double recessive Cream is relatively negative in its characteristics, both in regard to number and development of cells. The positive result of the dopa reactions suggests defective metabolism as a partial cause of the condition in the Cream type.

SUMMARY

The genetics of two new autosomal characters, Blond and Golden, in the guppy, *Lebistes reticulatus*, has been described.

The four phenotypes, Wild, Golden, Blond, and Cream, have been analyzed to show the differential effects of the various gene combinations on type, size, number, arrangement, and physiological reactions of the melanophores concerned.

The size, number, and arrangement of chromatophores in the Wild type appear to be in part the resultant of opposed tendencies of the two dominant genes.

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